ERRATUM

Anderson, H. H., and Anderson, J. D., Iodine Values and Lipids of Blood Sera, Volume 32, page 1473, last three lines should read, "Chaulmoogra therapy exhibited *lower* unsaturation of the fatty acids, but *higher* lipids . . . "

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8225 C*

Effect of Added Purines on Uric Acid Production by Isolated Tissues of the Rat.

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The effect of added purines on uric acid production by the kidney, liver, diaphragm, spleen, intestinal mucosa, and smooth muscle of the intestine of the rat was investigated by the methods previously described.[1]

The results (Table I) indicate that the intestinal mucosa and the liver account for most of the uric acid production from these purines. The mucosa is the only tissue that acts upon adenine to any extent.

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* P represents a preliminary, C a complete manuscript.

[1] Borsook, Henry, and Jeffreys, Cecil E. P., *J. Biol. Chem.*, 1935, **110**, 495

TABLE I.

Percentage conversion of added purines to uric acid by rat tissues in 4 hours at 37.5°C. in 5 cc. of glucose Ringer's solution containing 0.5 mg. purine (10 mg./%).

	Kidney	Liver	Diaphragm	Int. Mucosa	Smooth M.	Spleen
Adenine	9	5	1.5	85	—3.5	7
Guanine	15	78	10.0	69	13.0	17
Hypoxanthine	13	113	0.0	150	21.0	57
Xanthine	54	76	10.0	112	10.0	46

It is also very active in transforming guanine, xanthine, and hypoxanthine into uric acid. The liver actively transforms all these purines except adenine. Striated muscle (diaphragm) has very little effect upon any of them, and the small conversion due to smooth muscle may be the action of small quantities of mucosa which could not be removed. The kidney and spleen were only moderately active in the conversion of xanthine and hypoxanthine. The study of the production of uric acid in these tissues from other possible precursors is being studied further.

8226 C

Morphology of B. Acidophilus Grown in Soy Bean Milk.

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If *B. acidophilus* be inoculated to cow's milk and to soy bean milk, the microscopic picture of the two resulting cultures after 2 or 3 days' incubation is not the same. The growth in the usual cow's milk medium needs no description. All bacteriologists are well acquainted with it. A smear preparation of the culture grown in the soy bean infusion reveals many of the expected long rods with Gram positive tinctorial reaction. In addition to these rods of usual morphology, there are vast numbers of small units, likewise of Gram positive reaction and they may be of such small size that they approach the limits of visibility under the microscope. Mingled with these are varying numbers of organisms which are somewhat swollen and distorted and which are smaller also than the commonly described form of this microorganism. In the soy bean infusion culture, then, the numbers of long slender rods apparently are less than when the same bacillus is grown in cow's milk but the observer will be under the impression that there are far more bacterial units in the soy than there are in the latter preparation. This series of experiments was carried out in an endeavor to determine whether the small units present in the soy bean milk culture are indeed *B. acidophilus*.

We used a strain of the organism which originally had been sent from the laboratories of Dr. Kopeloff. The soy bean culture was checked most carefully both by aerobic and by anaerobic technique to assure ourselves that there was no contamination in it. A careful survey was made to select a medium among those which had been suggested for culture of *B. acidophilus* which should be most suitable for the plating of this particular strain. We chose the formula of Kulp and Rettger[1] which is entered as No. 2060 in the compendium of Levine and Schoenlein.[2]

Preliminary tests were made to determine by usual plating procedures whether *B. acidophilus* does multiply more rapidly in soy bean milk than it does in that from the cow. Each portion of medium was buffered by the addition of 0.5% calcium carbonate. Similar mass inoculation was made by pipette and incubation proceeded at 37°C. One representative curve from 3 done by us is now inserted.

TABLE I.

Comparative growth of *B. acidophilus* in soy bean milk and in cow's milk as determined by plating technique. Figures as millionms per cc.

Control	Soy bean	Cow's milk
(Initial)	15	12
6 hr.	15	12
8 "	225	30
20 "	900	115
32 "	845	80

Three possible methods of determining whether the small particles already described are living *B. acidophilus* occurred to us. One might endeavor to isolate a series by single cell technique. The exceeding minuteness of the forms precluded such an effort. One might be able to filter them through a Berkefeld candle but the consistency of the soy bean milk made such a procedure seem hopeless. The third possibility was to combine the technique of direct counting with plating. Breed and Brew[3] years ago elaborated the practice for direct microscopic counting of milk. The method seemed to be feasible to adopt and our plans developed as follows. Soy bean milk and cow's milk were sterilized separately in flasks in similar amounts. Approximately equal inoculums of *B. acidophilus* were then added by pipette. After shaking, incubation at 37°C. proceeded. At the expiration of each 6 or 8 hour period for a total time of 60 hours, a series of smears for direct examination was made and at the same time interval, a series of plate dilutions was poured with the medium already designated.

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[1] Kulp, W. L., and Rettger, L. F., *J. Bact.*, 1924, **9**, 357.

[2] Levine, M., and Schoenlein, H. W., A compilation of culture media for the cultivation of microorganisms, 1930. Williams and Wilkins Co., Baltimore.

[3] Breed, R. S., and Brew, J. D., *N. Y. Agr. Exp. Sta., Tech. Bull.*, 1916, **49**, 3.

From the smears made according to Breed and Brew's procedure, we computed the typically large forms of *B. acidophilus* per cc. of the different cultures at the times designated. Subsequent counting of the plates yielded information regarding the numbers of viable forms present in the same cultures regardless of the size of the organism which started the particular colony. This time consuming procedure was completed by us upon 2 different occasions. Results were similar in each instance. In Table II are shown figures obtained from one of these tests.

TABLE II.

B. acidophilus in soy bean milk. Comparison of total counts by plating and of direct microscopic counts of the large typically formed rods. Figures designate millions per cc.

hr.	Total count by plating	Large rods by direct count
24	295	145
30	310	179
36	265	179
44	570	79
52	555	106

The usual expectation when utilizing the Breed and Brew technique is that the total count of milk as indicated by plating will be fractional only of the enumeration by direct microscopic methods. The reverse is true, however, when these 2 operations are applied to *B. acidophilus* growing in soy bean milk and when the total plate results are compared to the numbers of the large supposedly typical microorganisms in that culture. We have already stated that the number of the small units in the soy infusion far exceeds the more usual morphologic type. Evidence thus is presented to indicate that the extremely microscopic form found here is also *B. acidophilus.*

Finally, we should add that when one stains a large series of the colonies appearing upon plates made from a culture of this organism growing in soy bean milk, smear preparations show only the large form usually described as *B. acidophilus*. It appears, therefore, that when the small forms grow upon the solid medium made up according to the formula indicated, they again take the usual type of morphology.

8227 C

Circulatory Changes During Recovery from Intense Exertion.

ERIC OGDEN AND N. W. SHOCK. (Introduced by J. M. D. Olmsted.)

From the Division of Physiology, University of California Medical School, and the Institute of Child Welfare, University of California, Berkeley.

Simultaneous measurements were made of pulse rate (continuous), systolic and diastolic blood pressures (½-minute intervals), pulmonary ventilation, oxygen consumption, and carbon dioxide elimination (10-20 consecutive samples) during the half hour following the fastest possible run upstairs, *viz.*, 17 meters (5 flights) in ½-¾ of a minute. The subjects were 20 young adults and 100 adolescent children—50 boys and 50 girls—and were resting supine while the measurements were made both in basal conditions before and also after the exercise.

Fig. 1 shows the result of a typical experiment. At first the pulse rate and pulse pressure were high and the diastolic pressure usually more or less low. The adults showed a maximum drop of 25 mm.

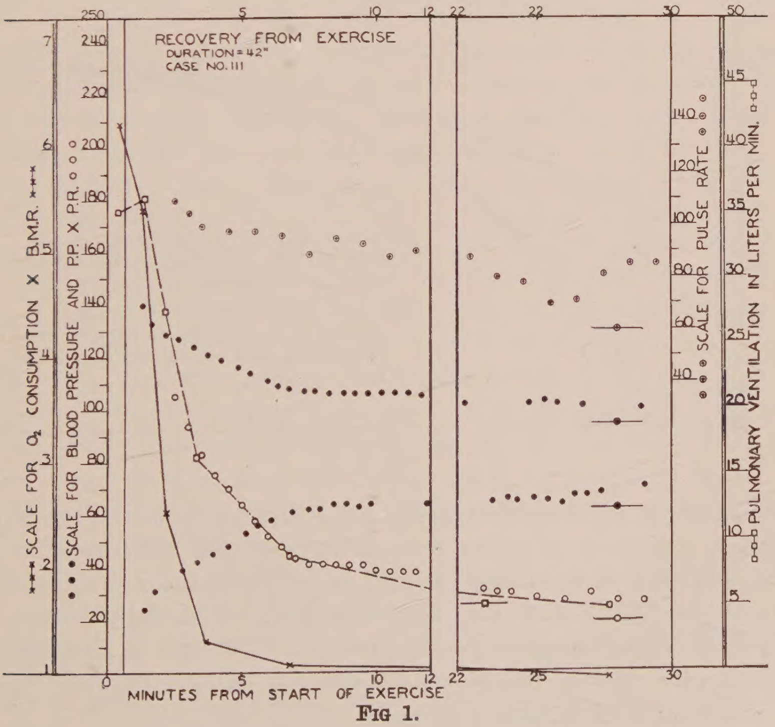
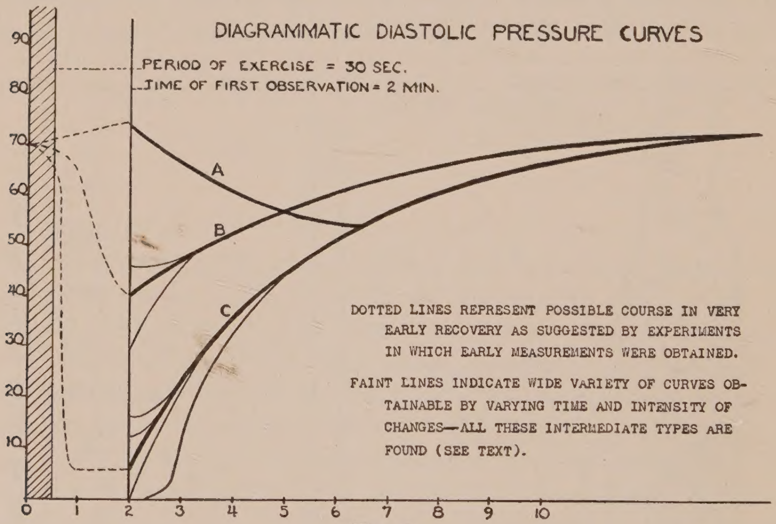


Fig 1.

Hg. and the children showed a maximum drop of 65 mm. Hg. below the basal value. Twenty-two percent of the children showed a drop of more than 25 mm. Hg., which was the greatest drop shown by any adult.

Low diastolic pressure with raised pulse rate and pulse pressure would appear to imply that arterial relaxation has taken place. The ensuing simultaneous decrease of pulse pressure and pulse rate indicates falling cardiac output (a phenomenon measured by Lindhard[1] in similar circumstances), and the simultaneous rise in the diastolic pressure which we observed can only have been accomplished by a vaso-constriction decreasing the peripheral resistance faster than the cardiac output dropped.

Variations in the time and intensity relations of these 2 vascular responses—relaxation followed by constriction—which appear to have been present in every case, can be held to account for all the types of diastolic pressure curves obtained on the basis of the 3 type curves shown in the diagram (Fig. 2). Although for convenience of discussion the diagram emphasizes 3 distinct types, all the intermediate varieties of curves were found between these types. All our data may be considered in one or other of these categories as far as the continuous lines are concerned. The dotted lines are hypothetical and suggest possible courses of events in the interval between our resting measurements and our earliest recovery meas-


DIAGRAMMATIC DIASTOLIC PRESSURE CURVES

PERIOD OF EXERCISE = 30 SEC.

TIME OF FIRST OBSERVATION = 2 MIN.

DOTTED LINES REPRESENT POSSIBLE COURSE IN VERY EARLY RECOVERY AS SUGGESTED BY EXPERIMENTS IN WHICH EARLY MEASUREMENTS WERE OBTAINED.

FAINT LINES INDICATE WIDE VARIETY OF CURVES OBTAINABLE BY VARYING TIME AND INTENSITY OF CHANGES—ALL THESE INTERMEDIATE TYPES ARE FOUND (SEE TEXT).

FIG. 2.

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[1] Lindhard, J., *J. Physiol.*, 1923, **57**, 17.

urements. For the most part, the adults fell into category *B* and the children into *A* and *C*, with a preponderance of boys in *C* and of girls in *A*. This would suggest that the adult vessels relaxed less markedly than those of the children and that among the latter, the boys' arteries reacted more rapidly than the girls'. This may be associated with our observation—to be reported separately—that the boys showed a more rapid rate of recovery of oxygen consumption than the girls. In view, however, of the notorious uncertainty of diastolic pressure measurements, the use of these conceptions in interpreting individual differences in diastolic pressure recovery curves must be reserved until more precise information is available on cardiac output in these circumstances, and until the relative speed of arterial response in these subjects has been measured by other techniques.

Estimates of cardiac output at the very beginning of the recovery from intense exercise have all been indirect. Lindhard showed a continuous diminution of cardiac output following work, but since this first computation depends on an oxygen consumption sample extended over a whole minute, he could not have observed changes in output during this time. Cotton, Rapport, and Lewis,[2] on the other hand, made early and frequent observations of pulse rate and systolic blood pressure from which they concluded that immediately after work the cardiac output fell approximately to its resting value, but mounted again during the ensuing half minute or so. The product of pulse rate and pulse pressure has been regarded[3, 4, 5] as a function of the cardiac output, and although the constancy of this function is uncertain, we believe the data in some experiments in which we obtained early measurements indicate that the cardiac output probably behaves more in accordance with Cotton, Rapport and Lewis' conception than with that of Lindhard, since in the experiments referred to, at the very beginning of recovery the pulse pressure × the pulse rate product is almost constant. However, our evidence was not obtained early enough in recovery to compare strictly with that of Cotton, Rapport and Lewis, and in view of the different varieties of diastolic pressure curves which we found, it would be interesting to see a repetition of their experiments with diastolic pressure measurements included.

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[2] Cotton, T. F., Rapport, D. L., and Lewis, Thomas, *Heart*, 1915-17, **6**, 269.

[3] Liljestrand, G., *Skand. Arch. f. Physiol.*, 1919, **37**, 180.

[4] Read, J. M. and Barnett, C. W., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 723.

[5] And others.

The data from these experiments show also that half an hour after the exercise, when the oxygen utilization of the children approached within 3-5% of its basal level, the pulse rate remained higher than basal and the pulse pressure, though variable, was usually lower than basal. The possibility of these phenomena being correlated with disturbances in the acid-base balance is suggested by the fact that the pulmonary ventilation is often above normal at this time and will be the subject of a later report.

Summary. 1. During recovery from exercise the relaxed arterial tree constricts. 2. The pulse pressure, and even the product of the pulse rate and pulse pressure, are not always decreasing in the first moments of recovery. 3. The basal circulatory equilibrium is not reestablished as quickly as is the basal rate of oxygen consumption.

8228 C

Resistance of Bacteria and Embryonic Tissue to Germicidal Substances. VI. Iodine Trichloride.

A. J. SALLE AND A. S. LAZARUS.

From the Department of Bacteriology, University of California, Berkeley.

Iodine trichloride (ICl_3) was first introduced as a germicide by von Langenbach.[1] It was described as a powerful disinfectant and recommended for the sterilization of hands, instruments and other surgical uses.

According to Hailer[2] the chemical is stable in concentrated solutions. In less concentrated solutions the iodine trichloride decomposes into iodine mono-chloride, iodic acid and hydrochloric acid, according to the equation, $2\ ICl_3 + 3\ H_2O \rightarrow HIO_3 + ICl + 5\ HCl$. In more dilute solutions the iodine monochloride decomposes to form iodic acid, free iodine and hydrochloric acid, $10\ ICl + 6\ H_2O \rightarrow 2\ HIO_3 + 8\ I + 10\ HCl$. The effectiveness of the compound is due probably to the amount of free iodine liberated.

In the first paper of this series[3] methods were described for comparing the resistance of bacteria and embryonic chick heart tissue to germicidal compounds. A *Staphylococcus aureus* phenol coefficient and a toxicity index were determined for each germicide tested.

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[1] Rideal, Samuel, and Rideal, Eric K., Chemical Disinfection and Sterilization, London, Edward Arnold and Co., 1921.

[2] Hailer, E., *Weyls Handbuch der Hygiene*, 1922, **8**, 861.

[3] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 665.

The highest dilution of phenol required to kill *Staphylococcus aureus* in 10 minutes but not in 5 minutes was 1:65. For iodine trichloride it was 1:6,000. This gave iodine trichloride a *Staphylococcus aureus* phenol coefficient of 92 by the method of Reddish.

Rideal and Rideal[1] reported that a concentration of 50 parts per million was required to destroy typhoid bacilli in 30 minutes. A saturated aqueous solution showed a phenol coefficient of 94. The activity of the compound was found to be only slightly impaired by the addition of albumin or salts. Behring[4] stated that iodine trichloride was a powerful disinfectant. He found that a 0.1% solution killed vegetative cells in one minute and a 1% solution destroyed spores within a few minutes.

The tissue culture results are summarized in Table I.

TABLE I.

Germicide	Highest Dilution Showing No Tissue Growth = A	Highest Dilution Showing No Growth of *Staph. aureus* = B	Toxicity Index = A/B	*Staphylococcus aureus* Phenol Coefficient
Phenol	1:840	1:65	12.9	
Iodine Trichloride	1:2,400	1:6,000	0.4	92

The results show that iodine trichloride, in addition to being a powerful germicide, is relatively very non-toxic to embryonic chick heart tissue. With the exception of iodine it showed the smallest toxicity index of any of the germicides so far studied when tested by the tissue culture technique. The compound showed more than twice the toxicity to *Staphylococcus aureus* than to embryonic chick heart tissue. Combining these results with those obtained in previous tests on other germicides,[3, 5–8] the chemicals may now be arranged in the following order on the basis of their toxicity indices: iodine = 0.09; iodine trichloride = 0.4; Hexylresorcinol = 3.0; Metaphene = 12.7; phenol = 12.9; Merthiolate = 35.3; and Mercurochrome = 262.0.

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[4] Behring, *Zeit. f. Hyg.*, 1890, **9**, 395.

[5] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 937.

[6] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1057.

[7] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1119.

[8] Salle, A. J., and Lazarus, A. S., PROC. SOC. EXP. BIOL. AND MED., in press.

8229 C

Influence of Temperature on Respiration of Cows.

M. KLEIBER AND W. REGAN.

From the College of Agriculture, University of California, Davis.[*]

The increase in the respiratory frequency as described by earlier workers for various animals, particularly for the dog,[1] has been observed in cows by Regan during his investigation on the influence of environmental temperature on milk production.

The data obtained with 2 cows indicate that the frequency of respiration is about doubled as the environmental temperature increases 10°C. By the method of least squares the results have been fitted to the Arrhenius equation:

$$\frac{f_2}{f_1} = e^{\frac{\mu}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right)}$$

where

f = frequency of respiration, breaths per minute
R = Gas constant, 1.985 cals per degree centigrade
T = environmental temperature in degrees Kelvin
μ = temperature characteristic[2]

The temperature characteristic thus calculated for the respiratory frequency was 11,980 cals for one cow and 13,070 cals for the other. The data with the respective regression lines are shown in Fig. 1. At environmental temperatures below 10°C. the influence of changes in temperature seems to be smaller than above 10°, which is probably not far from the critical temperature of milk cows during lactation.

The increase in the respiratory frequency with increasing temperature (above the critical temperature) is part of the animal's physical heat regulation. Particularly animals that do not have well developed sweat glands, as dogs and cattle, make use of polypnoea as a means of increasing evaporation of water for cooling. The question arose to which extent this mechanism for temperature regulation is operated ultimately by the environmental temperature at the body surface or by the temperature of the inspired air or both.

In order to answer this question a cow was kept in a large cham-

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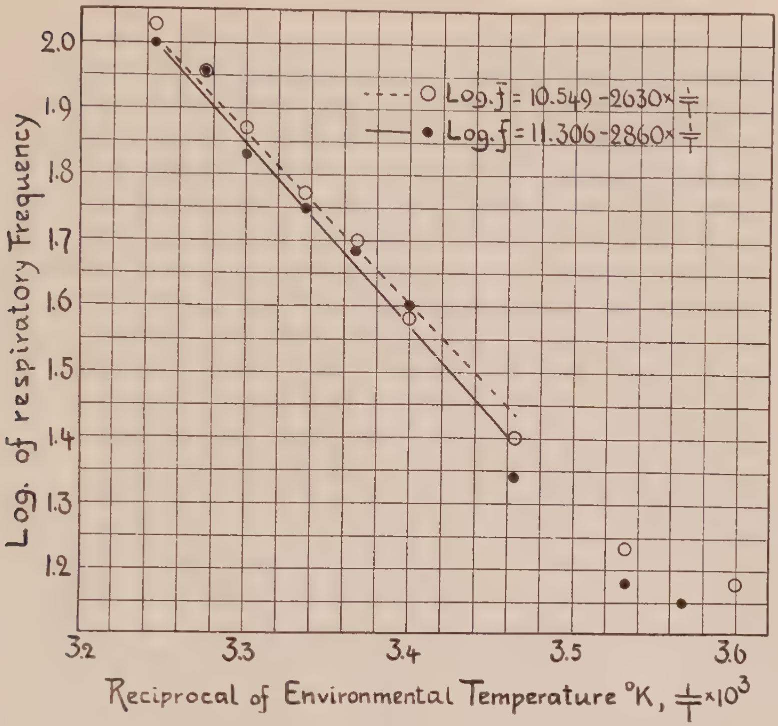
[*] The authors acknowledge the assistance of C. Winchester, S. W. Mead, and A. Folger.

[1] Review given by Bayer, *Handb. d. norm. u. path. Physiol.*, 1925, **2**, 272.

[2] Crozier, W. L., *J. Gen. Physiol.*, 1924, **7**, 123.



ber where the environmental temperature and humidity were controlled, the latter at about 50% saturation. The cow was breathing through a muzzle similar to that used by Brody[3] for respiration trials. The respiratory movements were recorded on a kymograph by an air buffer similar in principle to the "concertina" apparatus used by Haldane.[4] The amount of air expired was measured in a dry gas meter. Two air pumps (one at each end of the air duct), together with 2 valves operated electrically by a rubber bag manometer connected to the muzzle, helped to reduce the pressure against which the cow had to breathe.

Each experimental period lasted 10 minutes. During a first period the cow was allowed to inspire air from the chamber. For the second period the muzzle was connected to a smaller psychrometric cabinet regulated independently outside the main chamber. The cow inspired thus air which differed in temperature from that surrounding her body. The low heat capacity of the air and the heat exchange to the environment made it necessary to recondition the air in the muzzle itself. This was done in the first few trials by immersing the muzzle in a cooling mixture or a hot bath. The procedure was later improved by providing the muzzle with a rubber jacket, through which hot water or cold brine was pumped.

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[3] Brody, S., *Univ. Missouri Research Bul.*, 1930, **143.**

[4] Haldane, J. S., *Respiration*, 1927, 133.

Four trials have so far been completed with a low environmental temperature ranging from 6 to 9°C. and with a change of the temperature of the inspired air from 11 to 15°C. during the first period to a high level during the second period, ranging from 31 to 35°C. In 5 trials the cow was kept at an environmental temperature ranging from 32 to 37°C. The temperature of the inspired air was similar to that of the environment during the first period, subsequently the inspired air was cooled to a temperature ranging from 4 to 7°C.

TABLE I.
Frequency of Respiration.

	Breaths per Minute	
Temperature of Inspired Air		
Hot (31-36° C.)	15.4 ± 1.2	46.0 ± 3.8
Cold (4-15° C.)	15.5 ± 0.3	28.0 ± 2.0
	Cold (6-9° C.)	Hot (32-37° C.)
	Temperature of Environment	

Table I shows that the frequency of respiration was not increased when the inspired air was heated in a cold environment. The cooling of the inspired air in a hot environment, however, caused a marked decrease of the respiratory frequency. The random probability of this decrease is below 1%, the difference is thus statistically highly significant. The respiratory frequency was lower with the muzzle than without it. The data in Table I are, therefore, not directly comparable with those in Fig. 1, which were obtained without muzzle. Haldane[5] has shown in man that resistance in the air duct decreases the frequency of respiration. The resistance in our line during the experiment described here did not cause differences of pressure more than 2 cm. water level, yet at an environmental temperature of 35° we observed a drop in the respiratory frequency from 75 breaths per minute without the muzzle to 56 breaths per minute with the muzzle. When in a later trial the resistance was reduced so as to cause a pressure of approximately 5 mm. water level only, there was no marked difference in the counts of the frequency of respiration with or without the muzzle. In this trial the cow was breathing first cold air (11°C.) in a hot environment (38°C.) and then hot air (40°C.) in the same environment, the sequence of procedure was thus reversed from that followed in the earlier trials.

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[5] Haldane, J. S., *Respiration*, 1927, 49.

The respiratory frequency increased from 47.1 ± 1.3 breaths per minute when cold air was inspired to 72.6 ± 0.7 breaths per minute when the inspired air was as hot as the environment. This result thus checks the earlier findings and leads to the conclusion that in the trials with a difference of pressure up to 2 cm. water level, the effect of temperature on the respiratory frequency was superimposed on the effect of the resistance in the air duct.

TABLE II.
Rate of Pulmonary Ventilation.

	Liters$_{(S)}$ per Minute	
Temperature of Inspired Air		
Hot (31-36° C.)	55 ± 1.5	105 ± 5.4
Cold (4-15° C.)	52 ± 3.2	82 ± 7.2
	Cold (6-9° C.)	Hot (32-37° C.)
	Temperature of Environment	

The rate of pulmonary ventilation (Table II) was practically unaffected by raising the temperature of the inspired air in a cold environment. A considerable decrease of the rate of ventilation was observed, when the inspired air was cooled while the environment was hot (random probability 3.5%).

TABLE III.
Depth of Respiration.

	Liters per Breath	
Inspired Air		
Hot (31-36° C.)	3.6 ± 0.25	2.2 ± 0.04
Cold (4-15° C.)	3.4 ± 0.23	2.7 ± 0.14
	Cold (6-9° C.)	Hot (32-37° C.)
	Environment	

Table III shows the influence of temperature on the depth of respiration obtained by dividing the rate of ventilation into the frequency. The depth of respiration was not materially affected by the increase in temperature of the inspired air in a cold environment. The cooling of the air in a hot environment on the other hand increased the depth of breathing considerably.

The decrease in the depth of respiration may be important when polypnoea is a normal means of physical temperature regulation since it enables the animal to increase the rate of ventilation and yet not overventilate the alveoli, which would tend to lead to a depletion of CO_2 in the blood and thence possibly to apnoea. The effective ventilation for the exchange of CO_2 and O_2 is the tidal air minus the dead space. For the evaporation of water in the respiratory system, however, the whole tidal air is effective since the entire respiratory duct is lined with a moist membrane. By increasing the dead space per breath the animal can, therefore, increase the rate of pulmonary ventilation and hence evaporation without increasing the rate of alveolar ventilation. Shallow breathing as observed in polypnoea in our cow presumably increases the dead space per breath.

Summary. Increase in environmental temperature above 10°C. increased the respiratory frequency of 2 cows according to the Arrhenius equation with a temperature characteristic of 12 and 13 thousand calories respectively. Cooling the inspired air in a hot environment decreased the respiratory frequency as well as the rate of ventilation and increased the depth of breathing. Heating the inspired air in a cold environment did not significantly affect respiration. Shallow breathing at high frequency with increased dead space per breath enables an animal to combine a large total ventilation and evaporation of water with a relatively small alveolar ventilation.

8230 C

High-Titer Blood-Grouping Serum.

M. C. TERRY. (Introduced by W. H. Manwaring.)

From the Clinical Laboratory, U. S. Veterans Hospital, Palo Alto, California.

The importance of using only serum of high titer in determining human blood groups has been emphasized by Coca.[1] The risk involved in accepting a serum merely on correctness of type without attention to titer is shown by Coca's findings on examination of 16 sera in actual use in New York City, none of them coming up to his Grade I and only 4 to his Grade II.

Coca's standard for Grade I requires that a 1-4 suspension of cells be agglutinated macroscopically in one minute by a 1-4 dilution of the serum; by a 1-2 dilution for Grade II. Group "B" serum of acceptable titer (Grade II or better) is fairly common among persons of that relatively rare group. This cannot be said of Group "A", however. In either group a high-titer serum is the exception. It would seem, therefore, that a method for raising a low-titer serum to acceptable grade would be useful, particularly in the case of the usually low titer Group "A" serum.

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[1] Coca, Arthur F., *J. Lab. and Clin. Med.*, 1931, **16**, 405.

Our method consists in repeatedly freezing and thawing a tube of the serum in an ice and salt mixture and recovering the lower portion of the contents of the tube after the last thawing. An indication of what takes place may be seen if, between freezings, the tube is gently tilted back and forth, whereupon a movement of heavy oily-looking streaks occurs between a darker colored lower portion and a lighter colored upper portion. Ultimately the lower portion becomes deeply colored and the upper portion colorless and the lower portion will be found to have a higher agglutinin titer than the whole serum had originally.

By this method we have obtained from a serum which originally did not agglutinate macroscopically in 1-4 dilution a product which agglutinated in 1-16 dilution.

We are informed that this technic was employed in Wassermann's laboratory between 1908 and 1910. It has also been used in chemical research to reduce the water content of certain non-colloid solutions. We owe our acquaintance with the method to a note by Plant[2] who has shown that it can be used to obtain hemolytic complement of high titer from guinea pig serum. We have confirmed his findings as to complement and also as to rabbit serum anti-sheep hemolysin.

8231 C

Takata-Ara Reaction in Obstructive Jaundice.

J. L. CARR AND F. S. FOOTE. (Introduced by C. L. Connor.)

From the Departments of Pathology and Surgery, University of California.

The test that Takata[1] proposed was originally used to distinguish between lobar and lobular pneumonia and he reported the reaction with Ara[2] at this time on the serum in pneumonia and in cerebral spinal fluid as well, where it seemed to be of value in distinguishing between meningitis and luetic involvement of the central nervous system. The reason for the reaction was probably a protein-shift in the blood, according to most investigators and knowing that this occurs in many pathological conditions, Jezler,[3, 4, 5] Staub[6] and others employed the test upon serum or ascitic fluid in liver disease. They found that the test was positive in cases of advanced liver cirrhosis and also in cases of severe toxic parenchymal damage.

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[2] Plant, Arthur S., *Brit. Med. J.*, 1933, **2**, 414.

[1] Takata, M., Tr. 6th Congress, Tokyo, F.E.A.T.M., 1925, **1**, 693.

[2] Takata, M., and Ara, K., Tr. 6th Congress, Tokyo, F.E.A.T.M., 1925, **1**, 667.

[3] Jezler, A., *Z. f. klin. Med.*, 1929, **111**, 48.

Recently, Heath and King[7] have reported a series of over 400 cases with results which, in general, corroborate these findings. They have obtained positive tests of over 60% of cases of cirrhosis of the liver and also in cases of marked liver damage. They did not, however, get positive reactions in cases of biliary obstruction of the extra-hepatic type.

Having at hand 2 cases where a positive reaction was obtained from serum taken from cases of acute extra-hepatic biliary obstruction due to stones, we made observations on a series of dogs in which the extra-hepatic biliary system had been blocked by ligating the common bile duct. These animals, after operation, were given a balanced diet and blood was drawn weekly for examination. Of a total of 8 animals, 4 were obstructed by common duct ligation and 4 observed as controls. None of the 4 controls exhibited at any time a positive reaction either in serum or plasma. In the 4 animals jaundiced by occlusion of the common bile ducts, jaundice ensued within a few days, gradually deepening during the first 3 weeks, then maintaining a more or less constant level after this time. In 2 of the 4, the Takata-Ara reaction became positive the 45th day after ligating the common duct, in the third animal the reaction was positive on the 24th day, and the fourth showed a positive reaction 65 days after operation. These reactions were read as positive when there was a strong flocculation in 3 tubes, beginning with the second one, any less reaction than this being regarded as negative.

The survival period after development of the positive test was 54 and 148 days, respectively, in the 2 animals with a 45-day interval between operation and positive test, 33 days in the animal with a 24-day development period, and 128 days in the animal in which 65 days had elapsed from operation to positive flocculation.

By these figures it appears that a reaction becomes positive somewhere between one-half and one-third of the total time of survival after ligation of the common duct. It also appears that there is some relation between the rate of development of the test and the survival period.

Pathological changes in the liver at death were the usual and con-

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[4] Jezler, A., *Schweiz. med. Wchnschr.*, 1930, **60**, 52.

[5] Jezler, A., *Z. f. klin. Med.*, 1930, **114**, 739.

[6] Staub, J., *Schweiz. med. Wchnschr.*, 1929, **59**, 308.

[7] Heath, C. W., and King, E. F., *N. E. J. Med.*, 1934, **211**, 1077.

sistent findings with some definite increase in fibrous tissue about the periphery of each liver lobule and especially about the bile duct, a general infiltration of lymphocytes throughout this fibrous tissue and a little cloudy swelling of the cytoplasm of the liver cells.

Other liver function tests were done and many other observations on the blood serum carried out at this time, but the only parallelism existed between the Takata-Ara reaction and the sedimentation time which was materially reduced as the reaction became positive.

8232 C

Antigenicity of Streptofibrinolysin.*

J. K. VAN DEVENTER (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

The low antifibrinolytic titer† of most commercial anti-streptococcus serums[1] suggests the possibility that the specific antihuman fibrinolysin formed or secreted by certain strains of *Streptococcus hemolyticus* is not antigenic.

To test this possibility, active antifibrinolytic immunization was attempted with rabbits. The selected vaccines were (a) centrifugates from 24-hour broth cultures of fibrinolytic streptococci, and (b) lytic enzymes isolated from such cultures by the alcohol-precipitation technic.[2] Control injections were made with (c) heat-killed streptococci centrifuged free from lytic broth. There were also available for comparison (d) a series of rabbit precipitins for the Lancefield streptocarbohydrate A. This specific capsular sugar is apparently genetically linked with the antihuman fibrinolytic function.[3]

Three methods of immunization were used with these rabbits. Six animals (Group A) received 3 subcutaneous, 3 intraperitoneal and 3 intravenous injections at 3 to 4-day intervals, followed by 6 intra-

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* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† There is no known parallelism between the antifibrinolytic titer and the therapeutic value of a streptococcus antiserum. (W. H. M.)

[1] Van Deventer, J. K., PROC. SOC. EXP. BIOL. ANL MED., 1935, **32**, 1117.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[3] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 49.

venous injections at weekly intervals, the injection dose being: (a) 1,000 arbitrary units of the lytic enzyme, (b) 1 cc. (about 100 lytic units) of the centrifugate, or (c) equivalent amounts of lysin-free streptococci. Six rabbits (Group B) received the same vaccines intradermally (divided doses). Six animals (Group C) were given only one vaccine, the alcohol-precipitate, this was administered by means of a stomach tube, duodenal tube or enteric capsule.

Antiserums were withdrawn from all animals 10 days after the final vaccine dose. The average antifibrinolytic titer of each group of antiserums thus obtained is recorded in Table I.

TABLE I.

Antifibrinolytic Titers of Rabbit Antiserums

Each recorded number represents the average titer for the specified vaccine and animal group. The titrations were made with purified fibrinolysin, isolated human fibrin being used as the reaction index. For details of technic, see previous paper.[1]

Vaccine	Average number of arbitrary antifibrinolytic units[1] per cc. of antiserum			Normal rabbit serum	Normal human serum
	Group A	Group B	Group C		
(a) Centrifugate	80	80	—	30	60
(b) Alcohol-precipitate	80	80	80	40	50
(c) Heat-killed streptococci	80	80	—	30	40
(d) Carbohydrate A	30	—	—	20	80

Since none of the presumably immune rabbit serums had an antifibrinolytic titer more than 2 to 3 times that of normal rabbit serum, the conclusion seems justified that the antihuman streptofibrinolysin in itself is practically non-antigenic for rabbits.

It is conceivable that conjugation of the lytic factor with a colloidal "carrier" or even the establishment of an active focus of fibrinolytic infection may be necessary for the development of an effective antifibrinolytic immunity in this animal species.

8233 C

Passive Antifibrinolytic Immunity.*

J. K. VAN DEVENTER. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

As a preliminary to a study of passive antifibrinolytic immunity in laboratory animals, 5% high-titer commercial antistreptococcus serum† was added to freshly-drawn rabbit and rhesus bloods. The resulting passively immunized bloods were allowed to coagulate and serums separate. It was found that within the limits of the experimental error, the specific commercial antibody ("horse globulins") could be demonstrated quantitatively[1] in the resulting passively immunized serums, even after 30 days storage in the ice-chest.

Seven rabbits were then injected intravenously with 5% of their calculated blood volumes of the same antistreptococcus serum. Blood samples were withdrawn at the end of 15 minutes, 90 minutes and at stated intervals during the next 30 days. The serums from these samples were titrated for antihuman antistreptofibrinolysin, a scarlatinal strain of streptococcus and purified human fibrin being used in the technic. Ten percent of the same commercial antiserum plus 90% normal rabbit serum was used as the control. Composite data thus obtained are recorded in A, Fig. 1.

From this figure it is seen that the maximum humoral titer of the passively immunized rabbits never exceeded 60% of that of the test-tube control. Forty percent of the "horse globulin" was apparently bound, denatured or otherwise inactivated within 15 minutes after intravenous injection. This passive titer fell to 40% by the 11th hour, 10% by the 48th hour, and 5% by the 6th day.

The antistreptococcus serum selected for the above tests contains approximately equal amounts of 2 antifibrinolytic agents. One of these specifically neutralizes antihuman-rhesus streptofibrinolysin. The other is equally specific for antihorse streptolysin.[2] Passive im-

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* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

† The antiserums used in these and other tests were kindly furnished by Eli Lilly and Co., The Cutter Laboratory, Lederle Laboratories, E. R. Squibb and Sons, and Parke, Davis and Co.

[1] For titration technic see: Van Deventer, J. K., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1117.

[2] Madison, R. R., Proc. Soc. Exp. Biol. and Med., 1934, **32**, 444.

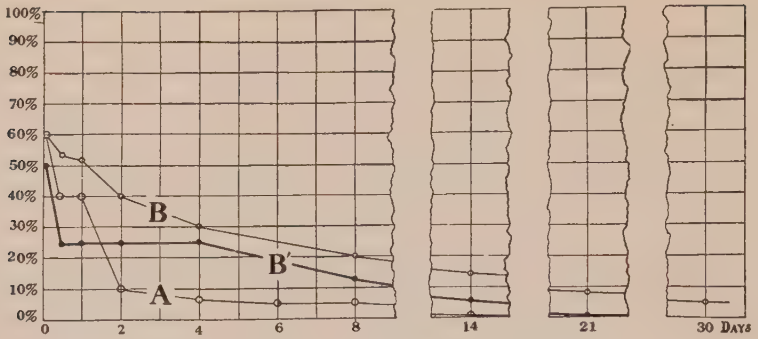

FIG. 1.
Intravenous Antistreptofibrinolytic Titer.

A, Rapid fall in heterologous passive antifibrinolytic serum titer in rabbits. Composite data from six rabbits; titrations with human fibrin; 100% represents calculated quantitative plasma retention of "horse globulins"; graph shows net titers obtained by subtracting the normal rabbit antifibrinolysin (2 to 4%).

B, Less rapid fall in homologous passive antistreptofibrinolytic titer in monkeys. Composite data from three monkeys; titrations with human fibrin.

B′, Parallel decrease in heterologous antistreptofibrinolysin in monkeys. Same blood samples titrated with horse fibrin. [The plateau effect in this graph is reminiscent of the apparent depolymerization of horse proteins in dogs.[3]]

munization of 3 monkeys was studied with both of these simultaneously injected antibodies, "horse-strangles" streptococci and purified horse fibrin being used in the veterinary titration. Composite data thus obtained are recorded in B and B′, Fig. 1.

From these graphs it is seen that the antibody homologous with human-rhesus fibrin (B), is bound, denatured or otherwise inactivated much more slowly in monkeys than in rabbits, about 5% of the intravenously injected "globulin" being demonstrable at the end of 30 days. The simultaneously injected heterologous antibody (B′), however, was reduced to 5% by the 14th day.

The different rates of decrease in these 2 simultaneously injected commercial "globulins" suggest a taxanomic differential between the rhesus-homologous and rhesus-heterologous specific antibodies of the horse, consistent with the Buchnerian theory of specific antibody synthesis.[4]

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[3] Sox, H., Azevedo, J. L., and Manwaring, W. H., *J. Immunol.*, 1931, **21**, 409.

[4] Manwaring, W. H., *J. Immunol.*, 1930, **19**, 155.

8234 C

Effect of pH on Heat Inactivation of Bacteriophage.*

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It has been shown by Krueger[1] and confirmed by Dreyer and Campbell-Renton[2] that the heat inactivation of bacteriophage follows the course of a monomolecular reaction. In Krueger's data the increase in rate of inactivation with rise in temperature was very rapid (μ in the van't Hoff-Arrhenius equation = 101,000). Critical thermal increments of this order of magnitude are uniquely characteristic of protein denaturation and the experimental results with phage were consequently interpreted as indicating that inactivation involved denaturation as a significant factor. The present paper deals with the effect of pH on the heat inactivation of the same anti-Staphylococcus phage used in previous work.

2.5 ml. of standard anti-Staphylococcus phage containing 1×10^{10} activity units per ml.[3] was added to 2.5 ml. of beef infusion broth containing sufficient normal HCl to bring the final pH to the desired point. The samples were placed in a water bath adjusted to 57°C. for one-half hour and the residual (Phage) determined by the activity titration method.[3]

Results are summarized in Table I and indicate that the phage suspension is most thermostable in the region of neutrality. At pH 7.0, 6.5, and 6.0 between 34% and 32% of the total original phage remains active; at pH 5.75 there is a sharp drop to 3% survival and at pH 5.5 to pH 4.0 less than 1% of activity is retained. On the alkaline side of neutrality the percentages of phage surviving the half-hour period of exposure to 57°C. are respectively: pH 7.5 = 50%, pH 8.0 = 24%, pH 8.5 = 10%, pH 9.0 and pH 9.5 = less than 1%. With the exception of the data obtained at pH 5.5 and pH 9.5 the experimental results are regularly reproducible within narrow limits. It is likely that the discrepancies noted at pH 5.5 and pH 9.5 are due to minor variations in pH since these points apparently represent zones of maximal thermolability and very slight differences in $[H^+]$ or $[OH^-]$ exert a considerable influence on the percentage of phage surviving.

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* Supported by Grants-in-Aid of Research from the National Research Council and the Board of Research, University of California.

[1] Krueger, A. P., *J. Gen. Physiol.*, 1932, **15**, 363.

[2] Dreyer, G., and Campbell-Renton, M. L., *J. Path. and Bact.*, 1933, **36**, 399.

[3] Krueger, A. P., *J. Gen. Physiol.*, 1929, **13**, 557.

TABLE I.

Effect of pH on Heat Stability of Phage.

Initial Phage = 5×10^9 Activity Units/ml.

pH	Log Phage Activity Units/ml. Remaining after Exposure to 57° C. 0.5 hr.		% Total Original Phage Surviving
9.5	<4.0	Aver. of 7 exp.	<1.0
9.0	7.6		<1.0
8.5	8.7		10.0
8.0	9.1		24.0
7.5	9.4		50.0
7.0	9.23	Aver. of 10 exp.	34.0
6.5	9.2		32.0
6.0	9.2		32.0
5.75	8.23		3.0
5.5	<7.0		<1.0
5.25	<3.0		<1.0
5.0	<2.0		<1.0
4.5	<2.0		<1.0
4.0	<2.0		<1.0

Controls consisting of Phage adjusted to all pH values from pH 9.5-pH 4.0 were kept at 20° C. 0.5 hour and then titrated. There was no loss of activity during this time.

It is known that hydrogen ion concentration markedly influences the rate of heat inactivation of many enzymes and it is commonly stated that hydrogen or hydroxyl ions catalyze the inactivation reaction. For example the observations of Morgulis and Beber[4] are explicable on this basis; they found that while the heat inactivation of catalase was slow at the iso-electric point, pH 6.0, it became very rapid at pH 4.0 to pH 5.0 and at pH 8.0 to pH 9.0. There seems to be no doubt, however, that additional factors are concerned in the heat inactivation of other enzymes. One special case of this sort is presented by trypsin, which is remarkably thermostable in dilute acid.[5] Crystalline trypsin has been proven to be a protein the denaturation of which is paralleled by loss of enzymatic activity[6] and resistance to high temperature is not a common property of either proteins or enzymes in solution. The explanation of trypsin's thermoresistance is to be found in the fact that as the solution is heated the protein is denatured and activity is lost, but upon cooling within the pH range obtaining in the experiment denaturation is reversed and enzymatic activity regained.[6]

The heat inactivation of phage has the high critical thermal increment characteristic of protein denaturation and with increases in $[H^+]$ or $[OH^-]$ the inactivation reaction is favored as in the case of many enzymes. The present data can be explained in two ways, first by postulating a simple catalysis of heat inactivation by hydrogen and hydroxyl ions and second by assuming that while Phage denaturation with corresponding loss of activity occurs at all pH values tested the reversal of denaturation and restoration of activity proceeds best within the pH zone close to neutrality. It is not possible to determine from the present experimental data which mechanism is actually concerned.

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[4] Morgulis, S., and Beber, M., *J. Biol. Chem.*, 1928, **77**, 115.

[5] Mellonly, J., and Wooley, V. J., *J. Physiol.*, 1913, **47**, 339.

[6] Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 323.

8235 C

V. Lymphatic Absorption in Simple Obstruction: Significance of Distention upon Its Occurrence.*

LOUIS SPERLING and OWEN H. WANGENSTEEN.

From the Department of Surgery, University of Minnesota.

It is generally conceded that the content of the obstructed bowel is toxic as is also that of the unobstructed gut. The matter of abnormal absorption from the obstructed bowel is still the subject of considerable debate and speculation. The possible avenues through which toxic material may be absorbed from the bowel are: (1) the mesenteric veins, (2) the lymphatics, and (3) transperitoneally by diffusion through the bowel wall. It has been well established by many investigators that under conditions of obstruction, venous absorption, at any rate for substances absorbed from the normal bowel, is decreased. Transperitoneal absorption apparently does not occur unless there is gross damage to the bowel wall by distention with impairment of its viability.[1]

In this study an attempt has been made to evaluate the occurrence of lymphatic absorption under conditions of simple intestinal obstruction and increased intra-enteric pressure. The absorption of dyes and of bacteria from the obstructed bowel was examined in the following manner:

Method and Results. Simple ileal obstruction was produced in 8 cats, and 20 cc. of 1% gentian violet or trypan blue was injected into

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* This work was supported by the Medical Research Fund of the Graduate School, University of Minnesota.

[1] Scott, H. G., and Wangensteen, O. H., Proc. Soc. Exp. Biol. and Med., 1932, **30**, 287; Sperling, Louis, and Wangensteen, O. H., Proc. Soc. Exp. Biol. and Med., 1935, **32**,

the bowel above the site of obstruction. Such animals succumbed after 24-120 hours. At autopsy, 3 showed colored lymph nodes. Closed ileal loops were prepared in 12 cats, and the loops were distended with dye until the walls became tense (marked increase of intra-enteric pressure). Ten of the 12 cats at autopsy (24-72 hours) showed blue nodes. In several of these cats as the dye was being injected under pressure into the lumen of the bowel, the dye could be seen after 5 to 10 minutes to outline Peyer's patches in the wall and later the lymph vessels in the wall; finally, with continued pressure the lymph vessels of the mesentery to the regional lymph nodes were visualized. This procedure was repeated *in vitro* with fresh excised viable specimens of normal and obstructed cat bowel with the following results: Pressures of 100 mm. of mercury in 2 normal loops forced the dye out into the lymphatics in 10 minutes. In another normal loop, a pressure of 50 mm. of mercury forced dye into the lymph vessels in one hour. When a fresh viable loop excised from a cat with simple ileal obstruction of 48 hours was subjected to 40 mm. pressure, the dye appeared in the lymph vessels in 30 minutes. When non-viable loops were subjected to increased intra-enteric pressure, the dye in addition to being forced rapidly into the lymphatics permeated directly through the bowel wall.

Fifteen normal control cats received varying amounts of dye by stomach tube and were operated upon at intervals of 24 and 72 hours. In none was the dye grossly evident in the mesenteric lymph nodes. Kagan recently has shown that the oral administration of trypan blue cut down the amount absorbed. When cats with simple ileal obstruction were given the dye by stomach tube, the dye did not appear in the lymphatics. Another control series (6 cats) was submitted to laparotomy, and a chemical peritonitis was induced with the intraperitoneal introduction of 5 cc. of tincture of iodine (to cause more profound intestinal stasis) and then the dye was administered by a stomach tube. At autopsy (24 to 72 hours) only one animal showed colored nodes. A third series of controls (5 cats) received the dye by enteric injection. The animals were operated upon again at intervals of 2, 5, 18, and 48 hours. In no case were the regional lymph nodes stained. At 5 hours, the dye had traveled from upper jejunum down into the colon.

The absorption of bacteria through the lymphatics was studied in the following manner: A heavy suspension of *B. pyocyaneus* was injected into the ileum of 6 normal cats. Preliminary control biopsy cultures of the regional lymph nodes made in several instances were negative. Cultures taken from the node one hour after intra-enteric injection were positive in 2 cases and negative in 4 for *B. pyocyaneus;* in 2 of the latter, positive cultures were obtained when the animals were reoperated upon after 24 hours. In 2 additional cats in whom the bacterial suspension was introduced into closed loops under pressure for one hour, the cultures from the regional nodes proved positive.

Similar experiments were carried out in normal and obstructed dogs. Positive cultures were obtained from the regional lymph nodes in 2 of 9 experiments on normal dogs, and in all of 6 experiments performed on dogs with low ileal obstruction of 24 to 96 hours' duration. Control cultures of the lymph nodes taken before injection of the bacterial suspension were negative in every case. Cultures of the thoracic duct lymph flow in these experiments on 5 normal and 6 obstructed dogs proved entirely negative. Cultures of femoral and portal (loop) blood in these cases were all negative.

In the experiments *in vivo,* the positive nodes of the closed loop were stained a definitely deeper color than those of the simple ileal obstruction group. The percentage of positive nodes was also much higher in the closed loop group than in the simple ileal obstructions. This was probably due to the fact that a higher intra-enteric pressure is developed in the closed loop.

In the control experiment, the absorption of dye into the lymphatics is no doubt prevented by its rapid transport through the bowel. Transport, of course, is abolished in obstruction. In its stead there is stasis, distention and increased intra-enteric pressure. From these experiments, we believe that there is a high-grade correlation between obstruction and the grade of intra-enteric pressure present, and the occurrence of lymphatic absorption. These findings are in accord with those of Costain,[2] Murphy and Brooks,[3] and Stone and Firor,[4] who have stressed the lymphatics as an avenue of absorption in obstruction. Williamson and Brown[5] had previously been unable to demonstrate in the lymph of the thoracic duct the presence of *B. prodigiosis* previously introduced into the unobstructed bowel. Ogilvie,[6] however, found that hemolytic streptococcus may be absorbed into the lymphatics and be cultured from the lymph. Hibbard[7] in this laboratory collected lymph from the thoracic duct after injecting 2% ammonium hydroxide with ammonia gas into a loop of jejunum. The ammonia content of the lymph was increased 4 times when the intra-enteric pressure was raised to 60 mm. of mercury. In 4 cats the lymphatic pedicle at the base of the mesentery was ligated without prolongation of the survival period attending the establishment of simple ileal obstruction.

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[2] Costain, W. A., *Surg., Gynec. and Obst.,* 1924, **38**, 252.

[3] Murphy, F. F., and Brooks, B., *Arch. Int. Med.,* 1915, **15**, 392.

[4] Stone, H. B., and Firor, W. M., *Tr. South. S. A.,* 1924, **37**, 173.

[5] Williamson, C. S., and Brown, E. I., *Am. J. M. Sc.,* 1923, **165**, 480.

[6] Ogilvie, W. H., *Brit. J. Surg.,* 1924, **12**, 752.

[7] Hibbard, J. S., personal communication.

It would appear that the regional lymph nodes normally serve as an effective barrier against overwhelming absorption of bacteria into the blood stream via the mesenteric lymphatic channels. A true bacteremia probably does not occur and most likely is not the cause of death in uncomplicated simple intestinal obstruction as we were unable to obtain positive cultures of the test organism from the thoracic duct. The lymph nodes yielding positive cultures acted as efficient barriers and precluded more centripetal invasion. A toxemia of bacterial origin, however, has not been wholly excluded.

Conclusion. Intestinal obstruction and increased intra-enteric pressure are conducive to increased lymphatic absorption of dyes and bacteria into the regional lymph nodes. No evidence, however, was adduced to indicate that this occurrence is of great significance in the lethal issue of bowel obstruction.

8236 C

An Improved Method for Determination of Blood Carotene.*

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Following closely upon the rapid accumulation of our knowledge regarding the relation of plant carotene to vitamin A, increasing interest has been manifested by investigators in the study of carotenoids. Thus far the investigations have been concerned chiefly with the carotene content of human and animal organism under different physiological and pathological conditions. The need of a suitable method for determining small amounts of carotene in the circulating blood has become quite apparent.

The methods hitherto employed[1] are more or less unsatisfactory in that they are impractical or non-specific. In some, fairly large amounts of blood are required for each estimation, which makes serial determinations difficult or impossible. Others are not specific and may lead to false results when they are used in the presence of other similarly reacting substances. The methods which involve the use of the yellow color of the carotin dye itself as an agent in the determination do not take into consideration that polyene dyes other than the carotenes, for example those of the xanthophyll type, may be part of the food intake and enter the blood. As a matter of fact, such instances were demonstrated by Palmer[2] and other investigators long ago. It is but natural, therefore, that under such conditions the yellow color of the blood serum extracts will represent the resultant of different dye substances, whereas the chief aim of all these methods should be the determination of the real carotene content of the blood. The present method was devised to eliminate the above-mentioned errors.

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*This research was aided by a grant from the Hungarian National Fund for Natural Science.

[1] Van den Bergh, H., Muller, P., and Broekmayer, J., *Biochem. Z.*, 1920, **108**, 279; Connor, Ch. L., *J. Biol. Chem.*, 1928, **77**, 616; Van Eekelen, M., *Acta brevia*

The carotenoids are precipitated completely from the blood serum by deproteination with alcohol. The dye is removed from the protein precipitate by repeated extraction with ether. After saponification of the lipoids and xanthophyll esters in the ether extract, the polyene hydrocarbons and xanthophyll dyes are separated from each other by dispersing them in a mixture of methyl alcohol and ether-petroleum-ether ("Entmischung"). The carotenes are contained in the upper layer, and may be determined in it colorimetrically.

Three cubic centimeters of the test serum are placed in a 20-25 cc. glass-stoppered centrifuge tube. After precipitation with 3 cc. of alcohol, the tube is carefully shaken and centrifuged. The supernatant fluid is discarded. The precipitate is repeatedly extracted with 3-4 cc. of ether. It is sufficient as a rule to repeat the extraction 4 times. To the combined dye-extract 0.5 cc. of a methyl alcohol solution of KOH (30%) is added; in order to prevent autoxydation, the liquid should be kept under nitrogen in an almost completely filled flask for about 24 hours. The fluid is then diluted with distilled water until separation into 2 layers takes place. An elongated graduated glass-stoppered separatory funnel of 25-30 cc. capacity proved to be the most convenient for this purpose. After

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Neerlandica, 1931, **1**, 3; White, F. D., and Gordon, E. M., *J. Lab. and Clin. Med.*, 1931, **17**, 53; Menken, D., *Med. Wschr.*, 1932, 1484; Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 804; Latest synopsis of Zechmeister, L., 1934, Carotinoide, and Bomskov, Chr., 1935, Methodik der Vitaminforschung.

[2] Palmer, L. S., *J. Biol. Chem.*, 1914, **17**, 191.

removal of the lower layer, the washing with water is repeated from 10 to 12 times, or until the wash water becomes neutral to litmus. As may be expected, the amount of ether is gradually decreased during this procedure, so that of the original ether used (9-12 cc.) only about half is left. To this washed ether extract one part of petroleum ether (50-60°) is added.

The separation of the polyene hydrocarbons from the oxygen-containing dyes now follows. By adding one volume of methyl alcohol (85%) to the above-mentioned dye-extract, the carotenes are recovered in the upper (ether-petroleum ether) layer, while the xanthophylls are held in the lower (methyl-alcohol) layer.[3] By removing the lower layer and washing the carotene-containing solution twice with fresh methanol, the xanthophylls are completely removed from it. It is important, however, to recover the small traces of carotene which may have been removed by the methyl alcohol. This can be done by re-washing the methyl alcohol extract with fresh petroleum ether. The upper layer is combined with the main ether-petroleum-ether solution, and the combined extract washed three times with 10 cc. of water to eliminate the last traces of methanol. This last treatment was found necessary, because the intensity of the color of the carotenoids is largely dependent upon the quality of the solvent.

The next step in the process is the evaporation of the carotene solution to dryness, *in vacuo* at 50°C. The ochre-colored residue is dissolved in 0.75-1.00 cc. of ligroin (70-80°), and the solution so obtained dehydrated with ignited sodium sulphate. Solution usually occurs in a very short time. The ligroin solution is poured into the trough of a microcolorimeter having a marked capacity of 1.0 cc. The deficiency is made up to the mark with ligroin rinsing of the evaporating flask. The entire carotene content of the original 3 cc. of blood serum is contained in this 1 cc. of ligroin.

The final colorimetric readings are made on the ligroin extract. For comparative purposes the azobenzene standard recommended by Kuhn and Brockmann[4] was employed: 14.5 mg. of azobenzene "Schering Kahlbaum" are dissolved in 100 cc. of 96% alcohol. The standard is set at 10 mm. Diffuse daylight proved to be most satisfactory. Five quick readings were made and the average taken. Control experiments did not differ more than 5% from each other. Known amounts of crystalline carotene added to the ether extracts of serum were recovered quantitatively.

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[3] Willstätter, R., and Stoll, A., *Untersuchungen über Chlorophyll*, 1913.

[4] Kuhn, R., and Brockmann, H., *Z. f. physiol. Chem.*, 1932, **206**, 41.

Experiments are now in progress to determine the merits of the method on a large scale in clinical cases.

We believe the method described here has sufficient accuracy to be applicable to small quantities of blood serum (3 cc.), and to serial determinations. The use of whole blood appears unnecessary. The investigation of Karrer and his collaborators[5] showed that the red blood corpuscles do not contain carotene. Special tests were carried out to ascertain whether, in the preliminary precipitation with alcohol, all of the blood carotene is precipitated. Tests were made also to determine whether the recovery of the dye by the extraction of the precipitate with ether is complete. For this purpose, 2 kinds of controls were employed. First, the fluid decanted from the alcohol precipitate was extracted with ether, and the extraction procedure repeated with ether and ligroin after the addition of lye; second, the precipitate itself was treated with 5% NaOH and extracted with ether several times. Neither the ether nor the ligroin used for the extraction contained any carotene.

In other experiments the effectiveness of the separation of carotenes from xanthophylls was tested on larger quantities of serum. For this purpose about 5 liters of mixed human blood serum was prepared in a manner similar to that described above for small quantities. After separation of the 2 solvent layers, the isolated dyes were identified with the aid of Tswett's chromatogram method and the grid-spectroscope.[6] For better identification of these dyes, a chromatogram (Tswett) was prepared from the ligroin solution of each isolated dye by pouring the solution through a $Ca(OH)_2$ column.† The results indicate that even some of the more recent investigators (Wendt[7]) have erred, basing their carotene determinations on the yellow color extracted directly from the serum; a considerable amount of the extracted material may belong to the xanthophyll type of coloring matter. The colorimetric determinations showed that the xanthophyll coloring matter present in the mixed human blood serum examined was about 15% of the total lipochrome content. In the chromatogram of the epiphasic dye a yellowish-pink ring developed above the yellow ring which is characteristic for carotenes; in the chromatogram of the hypophysis the zone characteristic of xanthophylls was seen. Each dye layer was dissolved separately in CS_2, and the extinction maxima determined with the grid spectroscope, with the following results. The upper yellowish-pink ring of the polyene hydrocarbon portion did not show any characteristic absorption band (autoxydized layer). The yellow ring below this gave an extinction maximum between those which are characteristic for A and B carotenes, *i. e.,* 514 and 479mμ. On the other hand, spectroscopic examination showed the main portion of the other fraction to be xanthophyll, with absorption bands at 509 and 473mμ (in CS_2).

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[5] Karrer, P., Euler, H. v. Hellström, H., and Rydbom, M., *Arch. for Kemi, Mineralogie och Geologie*, **10**, No. 12.

[6] Winterstein, A., and Stein, G., *Z. f. physiol. Chem.*, 1933, **220**, 247.

† Our thanks are due to Miss Njhelyi for valuable assistance.

[7] Wendt, H., *Klin. Wschr.*, 1935, No. 1.

In the experiment just described it appears evident, therefore, that the blood serum contains a considerable quantity of xanthophyll dye, which has to be eliminated from the test material before the carotene determination is made.

It is to be emphasized, however, that the method described may lead to false results in instances where the blood contains lycopene. Lycopene has been, as is well known, identified in human fat tissue[8] to which it is carried by the blood stream. Being a much more intense dye than carotene, a very small amount of lycopene is sufficient to cause an error in the colorimetric determination of carotene. The 5 liters of mixed blood examined by the writers contained no lycopene. It is important to keep patients whose blood is to be tested for carotene on lycopene-free (tomato-free) diet for several days before the blood examination.

Summary. Human blood serum contains other lipochromes besides carotenes, which must be removed from the test material before determinations of provitamine A are made.

8237 P

Homologous Function in Supernumerary Limbs After Elimination of Sensory Control.

PAUL WEISS.

From the Department of Zoology, The University of Chicago.

When in a salamander a supernumerary limb is transplanted into the neighborhood of a normal limb so that it receives its innervation from some part of the limb plexus, every movement of the transplanted limb is an exact duplication of the simultaneous movement of the nearby normal limb. This phenomenon of "homologous function," discovered by the author, has led to the conception of a "resonance" principle of nervous control.* Alternative explanations that had proved to be inadequate were (a) a strict selectivity controlling the establishment of nervous connections between the centers and the extra limbs, and (b) secondary central adjustments in the sense of "learning" or "conditioning", effected under the control of sensory messages reaching the centers from the extra limbs.

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[8] Zechmeister, L., and Tuzson, P., *Z. f. physiol. Chem.*, 1935, **231**, 259.

The former possibility has been ruled out by ample evidence. The latter possibility was contradicted by much circumstantial evidence, but a direct experimental test was wanting. Therefore, experiments were undertaken to decide whether or not homologous function would be found in limbs completely deprived of their sensory innervation so that the centers would fail to receive the necessary peripheral data prerequisite for the supposed conditioning or readjustment.

The experiments, performed on 10 axolotls of about 5 to 7 cm. in length, differed from the original ones in that, prior to the transplantation of the extra limb, the afferent nerve supply of the normal limb area into which it was to be transplanted had been thoroughly destroyed. The left fore limb was deafferented by extirpating the corresponding spinal ganglia 2, 3, 4 and 5, including their dorsal roots. I have reported[1] that, contrary to a widespread belief, the motor functions of limbs in amphibia are not noticeably affected by deafferentation, and that, despite the absence of tactile and proprioceptive control, the limbs continue to move in perfect coordination. The fact that the deafferentation in the operated specimens has been complete, was demonstrated in the living animals by the lack of sensitivity to mechanical stimulation in the deafferented limbs, and was confirmed later by the histological preparations proving the absence of spinal ganglia and dorsal roots throughout the operated area. One or 2 weeks after the deafferentation an additional fore limb was grafted to the deafferented shoulder region. Before inserting the transplant, part of the brachial plexus, now purely motor, was severed and assigned to the transplant for its nerve supply.

The first movements appeared in the grafts between 2 and 3 weeks after their transplantation, which, according to the earlier experiments with mixed nerves, is the time required for the regeneration of nerves into the transplants. *From their very first appearance, the movements exhibited clearly the phenomenon of homologous function,* each reinnervated muscle of the extra limb contracting synchronously with the corresponding muscle of the normal limb.

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* A short account of the "resonance" principle is given in the introduction to a recent paper by the author, *J. Comp. Neur.*, 1934, **61**, 135. A fuller description with a complete list of the literature can be found in DeSilva and Ellis, *J. Gen. Psychol.*, 1934, **11**, 145.

[1] Weiss, P., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 436.

The animals were kept under observation for many months, throughout which period the phenomenon of homologous function was observed as constantly and as strikingly as in those earlier experiments where the limbs had been in possession of their full sensory control.

It results from these experiments that sensory messages from the transplants are not instrumental in establishing homologous function. This is in agreement with the conclusions reached in the earlier work and embodied in the "resonance" conception, according to which the phenomenon of homologous function is a direct manifestation of a primary specific rapport between the impulses generated by the centers on one hand and the individual peripheral organs and their "modulated"[2] motoneurons on the other hand.

8238 C

Glycogen and Water Storage.

ESTHER M. GREISHEIMER AND EDITH GOLDSWORTHY.

From the Department of Physiology, The University of Minnesota.

MacKay and Bergman[1] stated that in rabbits there is a tendency for water to be stored in direct proportion to glycogen storage in the liver. The same authors[2] found that in young albino rats, on the average 3.8 gm. of water are stored with each gram of glycogen. That there is a relationship between glycogen and water storage is denied by Puckett,[3] Holmquist,[4] and Bridge.[5]

In view of the difference of opinion noted above, the authors decided to study the question. Adult rats (in most cases) of the Long-Evans strain were used. All the animals were kept on the McCollum Stock Diet I (whole wheat ground, 67.5%, casein 15%, whole milk powder 10%, butter fat 5.2%, calcium carbonate 1.5%, and sodium chloride 0.8%). The rats were killed by decapitation; the blood sugar was studied by the Shaffer-Somogyi method, the glycogen in a portion of the liver by the method described previously,[6] and the water content in the remaining portion of the liver by drying in an oven to constant weight at 105°C.

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[2] Weiss, P., *Anat. Rec.*, 1934, **60**, Suppl. p. 30.

[1] MacKay, E. M., and Bergman, H. C., *J. Biol. Chem.*, 1932, **96**, 373.

[2] MacKay, E. M., and Bergman, H. C., *J. Biol. Chem.*, 1934, **105**, 59.

[3] Puckett, H. L., and Wiley, F. H., *J. Biol. Chem.*, 1932, **96**, 367.

[4] Holmquist, A. G., *Skand. Arch. für Physiol.*, 1932, **65**, 9.

[5] Bridge, E. M., and Bridges, E. M., *J. Biol. Chem.*, 1932, **96**, 381.

In the first study 48 adult and 6 young rats were used. They were not fasted, but allowed the stock diet *ad libitum* until the time of killing. The results for the males and females are combined, and shown in Table I.

TABLE I.

	Liver Wt.	% Glycogen	% Water	Glycogen per liver (gm.)	Water per liver (gm.)	Blood Sugar (mg. %)	No. of rats
Min.	4.5946	2.17	68.65	0.183	3.23	73.63	
Max.	15.4192	6.76	70.92	0.917	10.79	113.47	
Aver.	9.3113	4.30	69.90	0.398	6.51	96.06	54

The relationship between glycogen and water content was tested by the determination of the correlation coefficient. When the percentage of glycogen and the percentage of water are used in the calculations, the correlation coefficient is +.202 ± .088, which would indicate a lack of correlation. However, when the absolute values, as glycogen per liver and water per liver, are used in the calculations, the correlation coefficient is +.745 ± .041. This indicates a significant relationship. Hence, in rats which have not been fasted, but allowed the stock diet *ad libitum* until the time of killing, the water is found to increase as the glycogen increases, by the method of random sampling.

In the same group of rats, other relationships were tested. When the blood sugar and the liver glycogen are studied, no relationship is found between the two. The correlation coefficient for blood sugar and percentage of liver glycogen is —.065 ± .091, and that for blood sugar and absolute glycogen is —.224 ± .087. We must conclude that in rats which have not fasted there is no relationship between the blood sugar and the liver glycogen.

Likewise, there is no relationship between the blood sugar and the water content of the liver, as shown by a correlation coefficient of +.050 ± .091 for blood sugar and percentage of liver glycogen, and —.154 ± .090 for blood sugar and absolute water.

In the second study, 43 rats were used. These were kept on stock diet until the night before killing; they were then fasted 12 hours, and subsequently divided into the following 5 groups. The rats in Group 1 were killed after the 12-hour fast; those in Group 2 were allowed stock diet *ad libitum* for 1½ hours before killing; those in Group 3, the same for 2½ hours; those in Group 4, the same for 3¼ hours, and those in Group 5, the same for 4 hours before killing.

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[6] Greisheimer, E. M., and Johnson, O. H., *Am. J. Physiol.*, 1929, **90**, 369.

The results for males and females are combined and shown in Table II.

TABLE II.

No. of rats	Liver Wt.	% Glycogen	% Water	Glycogen per liver (gm.)	Water per liver (gm.)	Blood Sugar (mg. %)
I (9a*)	7.784	0.315	69.69	.025	5.424	82.66
II (5a + 4y)	6.905	1.04	71.32	.066	4.926	109.38
III (4a + 4y)	6.177	1.67	70.99	.100	4.379	108.05
IV (4a + 4y)	7.264	2.47	70.93	.176	5.143	106.65
V (3a + 6y)	5.939	2.87	70.94	.168	4.199	109.82

* a = adults; y = young.

The authors did not find such close agreement as MacKay and Bergman[2] between the water storage and glycogen, in this study. However, the rats were not all of the same age, and they were fed stock diet instead of glucose and fructose. The time intervals varied in the 2 studies. This study does show that glycogen is deposited rapidly after feeding, and that it increases with the time elapsing between feeding and killing.

Summary. With a large group of rats taken at random, and kept on stock diet until killed, a high degree of correlation is found between the absolute glycogen and water content in the liver. With smaller groups, fasted for 12 hours, and fed the stock diet, then killed at intervals varying from 1½ to 4 hours, the relation between the glycogen and water content is less marked.

There is no correlation between the blood sugar and the liver glycogen, nor between the blood sugar and the water content of the liver.

8239 P

Gonadotropic Substance in Urine of Normal Children.

S. C. FREED. (Introduced by Samuel Soskin.)

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By utilizing the synergistic activity of certain pituitary extracts when combined with gonadotropic hormone, such as has been demonstrated on ovarian stimulation with pregnancy urine,[1] Evans[2] has been able to detect otherwise unappreciable amounts of gonadotropic substance in the urine of normal males. Because of this synergism and because it also resembled pregnancy urine, in causing a marked luteinization in the ovaries of his test animals, Evans referred to the gonadotropic substance in male urine as a "prolan body".

It has also been shown that a follicle stimulating substance, similar to the gonadotropic material occurring in the urine of castrates, is present in amounts of several rat units to the 24-hour excretion in the urine of normal adults, both male and female.[3] This follicle stimulating hormone has been reported to be absent from the urine of children before puberty.[3] We wish to report the finding of the "prolan" type of gonadotropic substance in the urine of children after the age of 4-5 years.

We employed the method of synergism referred to above. The urines of normal male and female children were extracted by the Katzman and Doisy benzoic acid method. A dose of this urine extract equivalent to 1/3-2/3 of a 24-hour excretion and administered over a 3-day period had no effect on the immature rat ovary at the end of 96 hours. Our pituitary extract was made with ammoniated alcohol, according to Evans,[1] and in the dosage used caused only a slight increase in ovarian weight of our test animals (an average increase of 6 mg. over the average control weight of 16 mg.).

When the 2 extracts were combined at the stated dosages a definite synergism, varying in magnitude with the age of the child, was observed. At the age of 4-5 years there was little or no effect. Above this age the gonadotropic response gradually increased until, at 10 years of age, values equalling those of normal adults were obtained

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[1] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **57**, 897.

[2] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **106**, 125.

(100-150% augmentation of ovarian weight with 1/3 of a 24-hour excretion). Histologic examination of the ovaries of our test animals revealed a heavy luteinization.

The presence of appreciable amounts of gonadotropic substance in urine of prepubertal children has been demonstrated. This hormone resembles "prolan" in its action and not the follicle-stimulating material found in castrate or menopausal urine. That it differs from the latter is also shown by the fact that, although Evans[4] has recently reported that the follicle-stimulating hormone of castration and menopause is synergistic with pituitary extracts, we were unable to obtain such effects with the pituitary extracts we prepared. Nevertheless this same pituitary preparation gave marked synergistic effects with pregnancy urine, as well as with the urine extracts of children as detailed above.

The presence of a prolan-like substance in the urine of normal children suggests the pituitary gland as its source.

I wish to thank Dr. Samuel Soskin for aid and direction.

8240 C

Some Effects of Ether on Bioluminescence in the Lampyrid, Photuris pennsylvanica.

GEORGE A. EMERSON. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville.

In normal anesthetic concentrations ether has no direct chemical action on the dehydrogenase system involved in carbohydrate metabolism in mammalian brain, but through humoral effects ether anesthesia in the intact animal has a marked action indirectly on the rate of autoxidation of subsequently excised surviving brain, due presumably to the limiting of available carbohydrate.[1] The present paper demonstrates a striking corollary in the *Lampyridae,* for while ether has little action directly on the dehydrogenase system having to do with bioluminescence, it does exert various indirect effects in the intact insect which yield again quite unexpected results.

The light-organ of *Photuris pennsylvanica* generally flashes spontaneously at irregular intermittent intervals. The characteristics of the flash have been studied photoelectrically by Snell[2] and the mechanism of control by Gerretsen, Snell and others,[2] it appears agreed that the flash is under nervous control. Access of adequate oxygen to the light-producing areas, which is a requisite for luminescence, is regulated by supposedly innervated[3] muscular tissue which normally between flashes almost completely occludes the entrance to tracheoles leading to the light-organ. Flashes may be artificially elicited through single shocks or short tetanic electrical stimulation, and the so-called pseudo-flash by rapidly raising the oxygen tension in an environment in which the oxygen concentration is low. Both artificial methods were found to be lacking in uniformity and to have potentialities of irreversibly injuring the flies, in confirmation of Snell's[2] work. Since no regular frequency of flashing can be induced in captured flies, it was obvious that little significant data could be obtained using flashing as a criterion for comparison of treated flies with controls.

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[4] Evans, H. M., and Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1047.

[1] Emerson, G. A., *J. Tenn. Acad. Sci.* In press.

Creighton's[3] observation, that injection of minute amounts of epinephrine hydrochloride into the fly results in an action on the occluding musculature of the tracheoles to permit the constant access of oxygen and a resultant constant bright glow of the light-organ, afforded an opportunity of preparing specimens for observation of the direct effects of gaseous agents on the luciferin-luciferase system *in situ.* The alternate dimming and brightening of the glow as observed by Creighton when the fly is alternately placed in atmospheres of nitrogen and oxygen was confirmed, and may be taken as evidence of the lack of the normal occluding mechanism, although both this and the postmortem anatomical evidence of action of epinephrine upon the tracheoles together are insufficient to answer the possibility of a direct chemical action of epinephrine upon the bioluminescence reaction. In lieu of experiments on an isolated luciferin-luciferase mixture which would show if epinephrine had any significant direct chemical effect, demonstration of the antagonistic action of the choline esters to the action of epinephrine and other sympathomimetic agents supports the hypothesis of indirect physical action in controlling the oxygen tension within the light-organ. Taylor's demonstration[9] of the effect of epinephrine HCl on bioluminescence in bacteria is suggestive but not absolutely conclusive of lack of action in an *in vitro* system of luciferin-luciferase. If a direct chemical effect exists, applications of Creighton's observations on the occluding musculature, some of the present results and those obtained on the California singing fish, *Porichthys notatus,*[4] would be modified or negated.

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[2] Gerretsen, F. C., *Biol. Zentralblatt,* 1922, **42**, 1; Harvey, E. N., *Physiol. Rev.,* 1924, **4**, 639; Snell, P. S., *J. Cell. Comp. Physiol.,* 1932, **1**, 37; *Science,* 1931, **73**, 372.

[3] Creighton, W. A., *Science,* 1926, **63**, 600.

[9] Taylor, G. W., *J. Cell. Comp. Physiol.,* 1932, **1**, 297; 1934, **4**, 329.

Flies prepared through intraabdominal injection of 0.01-0.02 ml. of 10^{-3} epinephrine hydrochloride were exposed to various concentrations of ether vapor in air, made up by Führer's[5] method. No immediate dimming of the constant bright glow was apparent except at the higher concentrations, so time of persistence of glow after 10 minutes' treatment with different tensions of ether vapor was chosen as a more sensitive criterion of action. Ten insects were used at each dosage level and the dimming end point arbitrarily taken as the time at which the bright glow, originally uniformly distributed over the 2 segments composing the light-organ, fell off so much that either of the segments appeared mottled or dark in spots when viewed through a 7 power hand lens. Results are given in Table I. Mortality was determined by examining the number of flies of 10 alive one hour after exposure for 10 minutes to ether, and motility of the insects by loss of posture on disturbing the anesthesia chamber by a sharp blow or shake, which normally induces vigorous running about. The mortality curve obtained by Knoefel and co-workers[6] on mice is

TABLE I.

Action of Ether on *Photuris* Treated with Epinephrine to Produce a Steady Glow.

	Epinephrine Treated			Untreated		
Conc. Ether mM/1	Av. Time of Persistence of Glow min.	Mortality Ratio*	Motility	Av. Time of Persistence of Glow, min.	Mortality Ratio*	Motility
0.0	>240	0/10	+	0	0/10	+
0.5	>240	0/10	+	0	0/10	+
1.0	>240	0/10	—	0	0/10	—
2.5	>240	0/10	—	w	0/10	—
4.0	>240	0/10	—	0.8	0/10	—
7.5	>240	0/10	—	5.2	0/10	—
10.0	178	2/10	—	6.1	0/10	—
12.5	152	3/10	—	8.1	1/10	—
15.0	144	8/10	—	11.8	4/10	—
20.0	0	10/10	—	†	10/10	—

*No. dead at the end of one hour after anesthesia/no. anesthetized.

†Glow extinguished during anesthesia; 2/10 glowed 14 min. after anesthesia.

w = weak glow present during anesthesia.

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[4] Green, C. W., and Green, H. H., *Am. J. Physiol.*, 1924, **70**, 691.

[5] Führer, H., *Biochem. Z.*, 1921, **115**, 235.

[6] Knoefel, P. K., *et al.*, *J. Pharmacol. Exp. Therap.* In press.

of interest for comparison, the corresponding MLD_{50} for 10 minutes' exposure being about 4.5 mM/1 of ether.

The data collected in Table I demonstrate that ether has little direct action in anesthetic and sublethal tensions on the dehydrogenase concerned with bioluminescence in the intact insect, even when no barrier to its accessibility exists in insects in which the light-organ tracheoles are opened through the action of epinephrine.

The indirect action of ether on bioluminescence in normal *Photuris* is also shown by Table I. Insects placed in ether vapor at concentrations from 1.0 to 4.0 mM/1 show a weak constant glow similar to that produced by intraabdominal injection of 10^{-7} epinephrine hydrochloride. At ether concentrations of from 7.5 to 15 mM/1, a constant bright glow involving the whole of both segments of the light-organ and of approximately the same intensity as the glow produced by higher concentrations of epinephrine results. Above 15 mM/1, the bright glow is not maintained through the period of anesthesia, but on removing the killed insects from the ether, a permanent soft glow slowly develops. In the light of Gerretsen's[2] findings on the action of chloroform on *Luciola vittata*, a Javanese lampyrid, and his explanation of the 3 stages of effect through simply the lethal action of the agent, it is of interest to note that motility is lost with ether concentrations below that effective on the light-organ tracheoles but that a maximal effect of ether is obtained at concentrations below lethal concentrations.

Creighton dismisses the theory that there may be humoral control of bioluminescence in the *Lampyridae*, although tissue histologically similar to that of the medulla of mammalian suprarenals has been demonstrated in invertebrates.[3] The effect of ether in producing a constant glow is a peculiar analogy to the probable mediation of many of the biochemical effects of ether by suprarenal activity in mammals,[1, 7] however, and strongly suggests that some such endocrine tissue may be active in this case also. At any rate, the actions of ether and epinephrine are in the same direction here as elsewhere. The results[8] obtained on lampyrids with other sympathomimetic compounds and with narcotics which may have an indirect sympathomimetic effect in mammals are also suggestive of true humoral control of the flash, as is the partial antagonism to such action by parasympathomimetic compounds injected previously. The pertinent pharmacology and its bearing on observations of Gerretsen,[2] Taylor,[9]

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[7] Emerson, G. A., *J. Pharmacol. Exp. Therap.*, 1935, **54**, 90.

[8] Emerson, G. A., and Knoefel, P. K. Unpublished.

and Shoup[10] on the effects of chloroform, urethane, epinephrine and other agents on various types of bioluminescence are discussed elsewhere.[8]

Summary. In the firefly, ether is effective in producing a constant glow through dilation of light-organ tracheoles only in concentrations well above the minimal anesthetic tension, while a maximum effect is produced at concentrations below the lethal tension; the effect of ether on bioluminescence is thus independent of both anesthesia and death. Ether inactivates the bioluminescence reaction *in situ* only at or above lethal tensions.

8241 P

Variations in Plasma Magnesium and Potassium in Epilepsy.

ARTHUR D. HIRSCHFELDER AND VICTOR G. HAURY.*
From the Department of Pharmacology, University of Minnesota.

McCollum and his collaborators,[1, 2] and also Greenberg and Tufts[3] have produced hyperirritability and convulsions in rats fed on a diet deficient in magnesium. Since we have found neuromuscular twitchings and convulsions in cases of clinical hypomagnesaemia,[4] since McQuarrie[5] believes that in epilepsy there is a "leakage of potassium" through the cell membrane, and since Hirschfelder[6] has shown that potassium salts can antagonize the narcotic action of magnesium, we have investigated the plasma magnesium and potassium in clinical epilepsy.

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[10] Shoup, C. S., *J. Gen. Physiol.*, 1929, **13**, 27; *J. Cell. Comp. Physiol.*, 1934, **5**, 269.

*Supported by a research grant from the Eli Lilly Co.

This investigation was conducted at the Minnesota Colony for Epileptics, whose clinical material and laboratory facilities were placed at our disposal by the Minnesota State Board of Control. To them, to Dr. D. E. McBroom, Superintendent of the Minnesota Colony for Epileptics, and to Dr. R. W. Brown we desire to express our thanks for their valuable cooperation.

[1] Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1932, **96**, 519; *Am. J. Physiol.*, 1932, **101**, 454.

[2] Kruse, H. D., Schmidt, M. M., and McCollum, E. V., *Am. J. Physiol.*, 1933, **105**, 635.

[3] Greenberg, D. M., and Tufts, E. V., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 674.

[4] Hirschfelder, A. D. (with the technical assistance of Victor G. Haury), PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 996; *J. Am. Med. Assn.*, 1934, **102**, 1138.

[5] McQuarrie, I., *Ann. Int. Med.*, 1932, **6**, 497.

[6] Hirschfelder, A. D., *J. Pharmacol. and Exp. Therap.*, 1929, **37**, 399.

We determined magnesium by the method of Hirschfelder, Serles and Haury[7]† and potassium and calcium by those of Kramer and Tisdall.[8, 9]

Total magnesium 1.7 mg. Mg or below 100 cc. plasma and ultrafiltrable Mg 0.7 or below were regarded as low; K above 30 mg. was regarded as high.

In epileptics whose plasma was collected during or just before convulsions we found the following deviations from normal: In 5 cases of status epileptics low magnesium (1.2 to 1.7 mg.) occurred in 4, high potassium (35 to 68.5 mg.) occurred in 5, high potassium/magnesium ratio occurred in 5. In 13 epileptics with very severe convulsions, low magnesium (1.3 to 1.7 mg.) occurred in 9, high potassium (31.7 to 63.6 mg.) occurred in 6, high potassium/magnesium ratio occurred in 13. In 12 moderately severe epileptics low magnesium (1.6 to 1.7 mg.) occurred in 3, high potassium (31.6 to 52.8 mg.) occurred in 8, high potassium/magnesium ratio occurred in 11. In 5 epileptics with mild convulsions, low magnesium (1.6 to 1.7 mg.) occurred in 2, high potassium (31.7 to 40.2) occurred in 3, high potassium/magnesium ratio occurred in 4.

Low plasma magnesium and high potassium therefore are very frequent in epileptic convulsions, and a high K/Mg ratio is the most frequent deviation from the normal. The ultrafiltrable K/Mg ratio rises much more than the total K/Mg ratio. Very low magnesium (1.1 to 1.4 mg., ultrafiltrable Mg 0.4 to 0.6 mg.) or very high potassium, (45 to 69 mg.), or both, were found in patients having the most severe convulsions. The greater the severity of the convulsions the greater was the deviation from the normal. However, there is no absolute critical level at which convulsions are certain to appear.

Plasma calcium was always within normal limits.

In 17 epileptics who were not having convulsions the plasma magnesium potassium and calcium were normal.

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[7] Hirschfelder, A. D., and Serles, E. R. (with the technical assistance of Victor G. Haury), *J. Biol. Chem.*, 1934, **104**, 647.

† Besides the standard solutions mentioned in our previous paper we used other proportionate dilutions of magnesium and Titan Yellow, so that the standard used for comparison corresponded more closely to the depth of color of the dye-containing diluted plasma.

[8] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **46**, 339.

[9] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1931, **48**, 223.

Oral administration of 2 gm. magnesium chloride 4 times per day for 3 months to 10 patients did not lessen the frequency of attacks, nor did 2 gm. potassium chloride 3 times a day for 3 months to 14 other patients increase the frequency.

8242 P

Cellulase from the Slug, Limax flavus Linnaeus.

WILLIAM W. TRIBBY AND EMMETT B. CARMICHAEL.

From the Department of Physiological Chemistry, School of Medicine, University of Alabama, University.

Seillière[1] and Billard[2] and others demonstrated the presence of cellulase in *Helix pomatia* by estimating the amount of glucose produced after action of the cellulase upon suitable substrates. Boynton and Miller[3] showed saccharification of wood by extracts from the ship-worm, *Bankia setacea.* Trager[4] found a cellulase from symbiotic intestinal flagellates of termites. He estimated the amount of glucose produced, both qualitatively (by means of Benedict's reagent) and quantitatively, after action of the enzyme upon filter paper which was dissolved and precipitated.

We also made use of the fact that reducing substances (carbohydrates) were produced, as proof for the presence of slug cellulase. Two substrates were employed: (1) A finely divided suspension of cellulose was prepared by dissolving 1 gm. of Whatman's No. 40 filter paper in 100 cc. of Cross and Bevan's $ZnCl_2$-HCl reagent. This solution was filtered by suction through cotton and poured into about 800 cc. of distilled water. The resulting precipitate was suspended in 400 cc. of distilled water and centrifugalized. This washing process was repeated ten times and the precipitate was finally allowed to settle out. (2) The outer coverings of date seeds were removed and discarded. Filings from the inner, pearly-white endosperm were put through a 100-mesh sieve and were either suspended in distilled water or weighed in the dry state for use as a substrate.

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[1] Seillière, G., *Compt. Rend. Soc. de Biol.*, 1910, **68**, 107.

[2] Billard, G., *Compt. Rend. Soc. de Biol.*, 1914, **76**, 566.

[3] Boynton, L. C., and Miller, R. C., *J. Biol. Chem.*, 1927, **75**, 613.

[4] Trager, W., *Biochem. J.*, 1932, **26**, 1762.

The slugs were killed with ether. Fifty animals were used. The gastrointestinal tracts and livers were dissected away from the bodies. Three different preparations were studied for cellulase activity: (1) The contents of the stomachs and anterior portions of the intestines. (2) The gut tracts, including the stomachs and intestines. (3) The livers. The second and third preparations were ground with 40-mesh sea sand and extracted with water or 0.7% NaCl for 72 hours. Cellophane was used as a membrane for dialyzing the gastrointestinal contents and extracts of the livers, to remove reducing substances that were originally present. Extracts of the gut tracts showed no reduction with Benedict's qualitative reagent.

The total volume of each reaction mixture was 3 cc. and consisted of 1 cc. of the solution to be tested for cellulase activity, 1 cc. of cellulose suspension or a weighed amount of cellulose and, either 1 or 2 cc. of buffer solution or water.

Both the fluid present in the gastrointestinal tracts and physiological saline (0.7% NaCl) or aqueous extracts of the livers gave positive reactions with Benedict's qualitative reagent after 2 to 8 hours, depending upon the concentration of the enzyme solution and upon the substrate used. With the precipitated filter paper, action did not proceed as rapidly as it did with the date endosperm cellulose. All reaction mixtures were kept in a dark place at room temperature and covered with toluene. No action was demonstrated by extracts from the walls of the stomachs and intestines.

Preliminary experiments indicated that the optimum pH for this cellulase is about pH 5.0 with acetate buffers.

Reaction mixtures containing 10 mg. of date endosperm in 3 cc. of solution (1 cc. of saline extract of liver, 1 cc. of acetate buffer pH 5.093 and 1 cc. of 0.7% NaCl) were run on 3 different liver preparations. The amount of sugar was determined by the Folin and Wu method; the reaction mixtures being treated the same as protein-free filtrates. Table I gives the per cent of sugar in the mixtures after 24, 48 and 72 hours.

TABLE I.

No. of Preparation	% sugar 24 hr.	% sugar 48 hr.	% sugar 72 hr.
1	.112	.143	.130
2	.070	.091	.110
3	.065	.082	.110

The extract used in preparation No. 3 was also allowed to act upon 20, 30, and 40 mg. quantities of substrate; the other constituents of the mixtures being the same as described above. The results are expressed in Table II.

TABLE II.

Amount of substrate	% sugar 24 hr.	% sugar 72 hr.
20 mg.	.135	.144
30 mg.	.202	.234
40 mg.	.270	.340

8243

Protective Action of Concentrated Antityphus Serum (Murine Type) Against European Typhus Virus.

HANS ZINSSER, M. R. CASTANEDA, AND F. D. HAGER.

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Two of the writers[1] have described a method of producing concentrated suspensions of Rickettsiae from peritoneal washings of X-ray radiated rats infected with typhus virus of the murine (Mexican-American) type. Such vaccines have since been used for human vaccination and for the production of antityphus serum by horse immunization. The reactions in the horse, the Weil-Felix reactions and the prophylactic and experimental therapeutic action of the immune horse sera have been elsewhere described.[2] Therapeutic test in man, which is giving encouraging results in Mexico, was justified by preliminary experiments in guinea pigs. In these experiments, moderate doses of the serum appeared to confer complete protection against the murine virus upon guinea pigs infected after, together with, or even 3 or 4 days before the administration of the serum. In the case of similar protection experiments in which the classical European virus was employed,[3] protection was not absolute, but sufficiently definite to encourage further efforts. Our observations of the close antigenic overlapping of the 2 principal varieties of human typhus Rickettsiae led us to believe that our difficulties, in regard to protecting against the European "humanized" virus, might be quantitative and could be overcome by increased potency of the antityphus horse serum. The present note reports work by which we believe the difficulty has been overcome.

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[1] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649; and PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

[2] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1933, **57**, 391.

[3] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1934, **59**, 471.

With the cooperation of the Massachusetts Antitoxin and Vaccine Laboratory, where our horse serum is produced, a method of concentration has been developed which has furnished a product which in moderate dosage protects guinea pigs against powerful injections of the European virus.

The method of concentration is, briefly, as follows: Typhus horse serum, produced as previously described (Weil-Felix test complete usually at about 1-320), is centrifuged in a Sharples centrifuge. It is then dialyzed in cellophane bags in running water to precipitate all insoluble protein (3 to 5 days). The precipitated protein is collected in a Sharples centrifuge and dissolved, so as to have a final content of NaCl 1%, phenol 0.2%, total solids about 12% to 13%. This solution is then clarified in the Sharples centrifuge and filtered through an N Berkefeld candle.

Preliminary observations showed that the Weil-Felix antibodies were concentrated in the sediment of such dialyzed serum, and subsequent protection tests have demonstrated that protective antibodies are similarly precipitated. Complete analysis of the results of concentration will be published more fully elsewhere, but in a number of cases, from an original volume of somewhat less than 5 liters of a weak serum having a Weil-Felix titre of 1-160, 700 cc. of concentrate has been obtained, which gave a powerful Weil-Felix in a dilution of 1-1280 and a partial Weil-Felix in a dilution of 1-2560. Two series of protective tests with such concentrated serum against the European virus have been carried out as follows:

In each set, the European virus—in the form of 2 cc. of fresh blood, in the first experiment; and 2 cc. of a defibrinated blood-brain mixture, in the second experiment—was injected intraperitoneally into a series of 7 guinea pigs. One animal in each case was kept as a virus control. In the first experiment, 3 hours after intraperitoneal inoculation with virus, 3 animals received, subcutaneously, 1 cc. of antityphus serum concentrate. Three others received, by the same route, 1 cc. of a similarly concentrated antipneumococcus serum. In the second experiment, the serum was given 24 hours after virus injection, in similar amounts and again subcutaneously. The controls, with a concentrate of known specific immune serum, were made in order to insure against accidental phenomena due possibly to non-specific protein factors or other unfavorable effects.

The consolidated charts show the results.

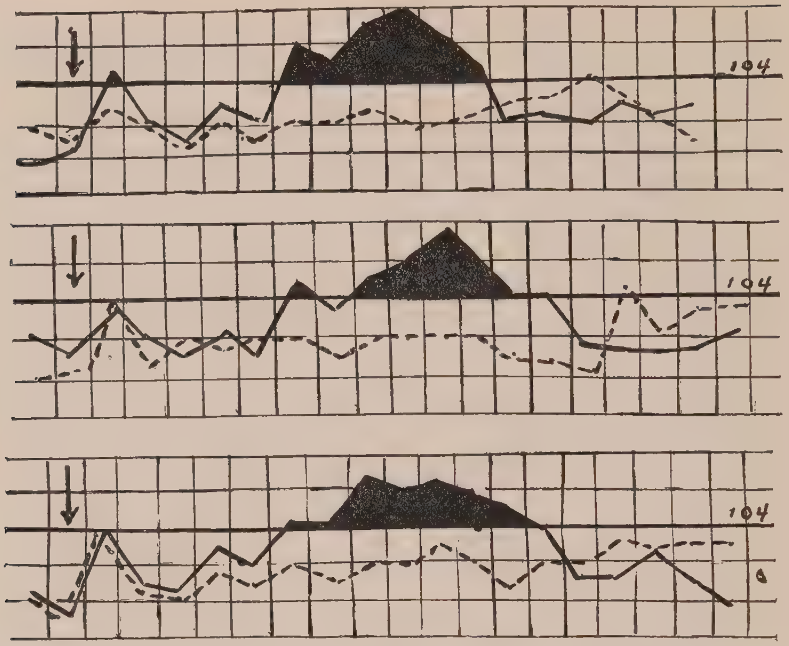

FIG. 1.

Charts of Guinea Pig Temperatures of Second Experiment.

Control without serum is omitted, but ran the typical course of European guinea pig typhus. All the 6 animals charted received on the first day an intraperitoneal injection of 2 cc. of defibrinated blood and brain emulsion mixtures of a passage animal, the materials being taken on the tenth day of the disease at a time when the temperature was almost 105°. 24 hours later, at the points shown by arrows, 3 animals, whose temperature curves are shown by the broken line, received, subcutaneously, 1 cc. of concentrated antityphus serum, as described. The 3 controls, whose temperature curves are shown by the uninterrupted line, received, subcutaneously, 1 cc. of similarly concentrated antipneumococcus serum. Temperatures above 104° are blocked in black.

It will be seen that the serum concentrate, although obtained from a horse immunized entirely with Rickettsiæ of the murine Mexican type, protected satisfactorily against powerful doses of European virus, even when the virus was given a considerable advantage by intraperitoneal injection and a 24-hour start.

In the first experiment, the virus control showed a typical curve of European typhus of ordinary severity. The animals which had received the concentrated antipneumococcus serum all passed through a typical attack of typhus, temperatures rising on the 7th and 8th days and following the usual 7 to 8-day fever characteristic of this disease in guinea pigs. The 3 animals which had received the antityphus concentrates showed no reactions except that in one animal, on the 13th day, the temperature rose to 104.2°, promptly falling again to 103° on the following day. In the second experiment, in which the dosage of virus was much more severe, owing to the admixture of typhus brain suspension with the defibrinated blood, the result was identical.

In previous experiments it was found that unconcentrated antityphus horse serum, while highly efficient in protecting guinea pigs against the Mexican murine virus, was only partially effective in similar experiments carried out with European humanized virus. The results of these experiments, added to observations of serological overlapping encountered in active immunization and in various types of agglutination tests, indicated that a possible increase of potency might render a serum produced by immunization with the murine Rickettsiae effective against the virus of the classical European disease. The experiments outlined demonstrate that this is the case. Concentration on a practical basis, by a method outlined, has yielded a product of which 1 cc. administered subcutaneously after infection will completely protect guinea pigs against considerable doses of intraperitoneally administered European or humanized virus. The gradual absorption known to take place after subcutaneous injections of serum gave the virus a considerable advantage. The method was used in order to simulate more closely actual therapeutic conditions. The production of sufficient quantities of such serum concentrate for therapeutic test in the classical European typhus is not prohibitive either in time or work for practical application.*

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* Since these notes were prepared for publication, an observation has been made in the laboratory which is well worth recording, though not deserving a separate communication. A worker, ''M,'' ran a needle on which was balanced a heavily infected bit of tissue culture, loaded with Rickettsiæ, into his thumb. He had been vaccinated with our murine typhus vaccine four months previously, but had no Weil-Felix reaction. He was given 5 cc. of a concentrated antityphus horse serum on the day of this accident (Weil-Felix of serum, 1-2480) and 10 cc. forty-eight hours later. Our own nervousness accounts for the probably unnecessary high dosage. Since the simple washing with iodine immediately practiced probably had little effect, we feel that we should at least record the fact that outside of mild serum sickness, ''M'' has remained entirely well. No conclusions can of course be drawn from this, but in connection with the experiences of Bustamante and Varela with prophylactic use of our serum in Mexico, the experience is worth recording and emergency serum injections are recommended to other workers.

8244 C

Experimental Goat's Milk Anemia.

HOWARD L. ALT.

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Many reports have appeared, especially in the German literature, concerning a pernicious anemia-like syndrome occurring in infants fed with goat's milk.[1] György[2] has recently reported such cases which responded to liver but not to iron therapy. Rominger, Bomskov, *et al.*,[3] have reported the occurrence of a pernicious anemia picture in young white rats fed on goat's milk. The erythrocyte counts decreased rapidly without corresponding decreases in the hemoglobin. This anemia did not respond to iron therapy but responded to liver extracts effective in pernicious anemia. Von Haam and Beard[4] also have observed a hyperchromic character of the anemia in rats during the first weeks of feeding with goat's milk. György[2] states that he was unsuccessful in producing a hyperchromic anemia in rats by this method. The experiments reported in this paper show no evidence of a specific type of anemia in white rats fed on goat's milk.

Three litters of 6 albino rats each, of the Wistar strain, were weaned at 23 days and put into individual glass cages. Their weights averaged 40-45 gm. They were fed exclusively on goat's milk* which was collected directly into clean glass containers at the dairy and delivered to the laboratory every other day. Erythrocyte counts were performed with Bureau of Standards equipment and the hemoglobins with the Newcomer apparatus standardized by the oxygen capacity technique. Erythrocyte counting pipettes and micro-colorimeter cups were used in the hemoglobin determinations. Reticulocyte counts were performed by the method of Cunningham.[6] Cover slips containing brilliant Cresyl blue and blood were allowed to be in apposition several minutes before making the smears in order to insure complete staining of the reticulocytes.

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[1] Korteweg, R., *Nederl. Maandschr. V. Verlosk, Vrouwenz, enz.*, 1916, **5**, 337, and Scheltema, G., *Nederl. Maandschr. V. Verlosk, Vrouwenz, enz.*, 1916, **5**, 407 (Reported by Brouwer); Kleinschmidt, H., *Jahrb. f. Kinderh.*, 1916, **83**, 97; Schwenke, J., *Jahrb. f. Kinderh.*, 1918, **88**, 181; Stoeltzner, W., *Munch. Med. Wchschr.*, 1922, **69**, 4; Blühdorn, K., *Munch. Med. Wchschr.*, 1922, **69**, 1220; Brouwer, E., *Jahrb. f. Kinderh.*, 1923, **102**, 257, 357; **103**, 51; Beumer, H., and Wieczorek, G., *Jahrb. f. Kinderh.*, 1924, **107**, 311; Opitz, H., *Jahrb. f. Kinderh.*, 1925, **108**, 311; Stettner, E., *Monatschr. f. Kinderh.*, 1925, **29**, 587; Ockel, G., *Jahrb. f. Kinderh*,. 1925, **110**, 62; Glanzmann, E., *Jahrb. f. Kinderh.*, 1926, **111**, 127; Grävinghoff, W., and Neuhaus, C., *Monatschr. f. Kindh.*, 1928, **40**, 71; Baar, H., *Jahrb. f. Kinderh.*, 1928, **120**, 242; Hyland, C. M., *Arch. Pediat.*, 1929, **46**, 673; Letterer, E., *Jahrb. f. Kinderh.*, 1930, **130**, 1; Haase, G., *Monatschr. f. Kinderh.*, 1934, **60**, 241; Von Haam, E., *South. Med. J.*, 1935, **28**, 22.

[2] György, P., *Z. f. Kinderh.*, 1934, **56**, 1.

[3] Rominger, E., Meyer, H., and Bomskov, C., *Z. f. d. ges. Exp. Med.*, 1933, **89**, 786; Bomskov, C., and Auffarth, M., *Z. f. d. ges. Exp. Med.*, 1933, **89**, 804; Bomskov, C., and Czerlinsky, H., *Z. f. d. ges. Exp. Med.*, 1933, **89**, 809; Rominger, E., and Bomskov, C., *Z. f. d. ges. Exp. Med.*, 1933, **89**, 818; Rominger, E., and Bomskov, C., *Klin. Wchschr.*, 1935, **14**, 148.

[4] Von Haam, E., and Beard, H. H., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 750.

This series of 18 rats received a goat's milk diet for a period of 65 days. The average erythrocyte counts increased gradually from an initial level of 6.8 million to a level of 9.5 million. The average hemoglobin values decreased from 14 gm. to 8 gm. per 100 cc. The reticulocyte percentage at the beginning averaged 5%, increased to 13% in about 10 days, and then decreased. There was a gradual secondary rise reaching to about 10% at the end of the experiment. These results are similar qualitatively but slightly less marked than those observed with cow's milk in this laboratory.[7] The slowness with which the anemia appeared might have been related to storage of hemopoietic materials before weaning since the mothers received meat and vegetable feedings once a week in addition to the regular diet.†

In an attempt to obtain a more severe anemia in a shorter period of time, the method of Elvehjem and Kemmerer[8] was employed. Two litters of 6 rats each were placed in separate glass cages with the mothers 2 days after birth. The mothers were removed for about 4 hours daily and given the stock diet. Goat's milk was available in the glass cages at all times. At 21 days, the young were weaned and were given an exclusive diet of goat's milk. Their average weights at this time were 30-35 gm. The erythrocyte counts averaged from 3 to 5 million and the hemoglobins from 7 to 8.5 gm. per 100 cc. During the next 3 weeks of goat's milk feeding, the erythrocyte counts increased slightly, whereas the hemoglobin values decreased to about 4.5 to 6 gm. per 100 cc. (Table I.)

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* The goats were of the Alpine type and received complete diets. Analyses of samples of milk were made for copper by the method of Elvehjem and Lindow.[5] Typical results of analyses on different samples of milk: Copper, mg./liter, .20, .20, .23.

[5] Elvehjem, C. A., and Lindow, C. W., *J. Biol. Chem.*, 1929, **81**, 435.

[6] Cunningham, T. D., *Arch. Int. Med.*, 1920, **26**, 405.

[7] Farmer, C. J., and Cory, H. E., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 766, and personal communication.

† One-third whole milk powder and two-thirds whole wheat, containing 1% iodized sodium chloride.

[8] Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

TABLE I.

Development of Anemia in Rats Receiving Goat's Milk.

Rat No.	Time in Days	R.B.C. in mill./cc.	Hgb. in gm./100 cc.
224	0	3.6	6.8
	7	4.2	5.8
	14	—	6.8
	21	6.7	5.9
227	0	4.9	8.0
	7	4.5	7.4
	21	5.1	4.8
230	0	3.1	8.5
	14	—	4.5
	21	5.2	4.5

In order to study the effect of anti-anemia therapy, these rats were divided into 3 groups. Group 1 received daily supplements of iron and copper. Group 2 received liver extract containing the anti-pernicious anemia factor‡ plus an iron supplement, and group 3 received an extract containing the hemoglobin-producing factor of Whipple.[9]§ All 3 groups received equivalent amounts of copper while the iron content of the diet containing extract 55 was somewhat higher than the rest. The rats were studied over a 2-week period, and the results are recorded in Table II. The increases in the erythrocyte counts and hemoglobins per 100 cc. were similar in all the animals regardless of the type of therapy. It is apparent that the rats receiving liver extract gained about 2.5 times as much weight in the 2 weeks as the rats which received only the iron and copper supplement. The presence of a growth factor in liver has been reported previously.[11]

The results in general are similar to those that might be expected from the feeding of cow's milk. A hypochromic anemia

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‡ Liver Extract 343, Eli Lilly and Company.

[9] Whipple, G. H., Robscheit-Robbins, F. S., and Walden, G. B., *Am. J. Med. Sc.*, 1930, **179**, 628.

§ Liver Extract 55, Eli Lilly and Company.

These liver extracts were analyzed for iron by the method of Hanzal[10] and for copper by the method of Elvehjem and Lindow.[5]

[10] Hanzal, R. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 846.

[11] Kline, O. L., Elvehjem, C. A., Keenan, J. A., and Hart, E. B., *J. Biol. Chem.*, 1934, **107**, 107.

TABLE II.
Effect of Various Therapeutic Measures on the Anemia from Goat's Milk.

Rat No.	Time in Weeks	R.B.C. in mill./cc.	Hgb. gm./100 cc.	Weight in gm.
Group I.				
Daily Supplement: 0.5 mg. iron (as $FeCl_3$), 0.04 mg. copper (as $CuSO_4$).				
225	0	4.4	4.7	65
	2	9.6	12.7	77
226	0	5.2	6.2	59
	2	9.3	14.8	71
231	0	4.5	5.1	72
	1		12.9	
	2	6.9	12.7	93
232	0	5.7	4.6	71
	1		11.5	
	2	7.3	13.7	85
Aver. increase 2 wks.		3.3	8.3	15
Group II.				
Daily Supplement 1 gm. Liver Extract 343 (contained .139 mg. iron, .043 mg. copper), 0.35 mg. iron (as $FeCl_3$)				
223	0	4.8	4.7	66
	2	8.7	15.2	108
224	0	6.7	5.9	59
	2	10.1	15.6	91
229	0	5.1	5.1	71
	1		11.9	
	2	9.5	13.9	103
230	0	5.2	4.5	78
	1		12.0	
	2	9.0	14.1	125
Aver. increase 2 wks.		3.9	9.6	38
Group III.				
Daily Supplement: 1 gm. Liver Extract 55 (contained 3.30 mg. iron, 0.039 mg. copper.)				
221*				
222	0	6.3	8.4	57
	2	8.9	13.4	98
227	0	5.1	4.8	73
	1		13.1	
	2	9.2	14.5	108
228	0	4.6	4.3	77
	1		13.0	
	2	8.7	15.3	113
Aver. increase 2 wks.		3.6	8.6	37

*Data omitted because rat obtained food.

occurred from the exclusive feeding of goat's milk, which showed a maximum response to iron and copper therapy. The difference in these results, as compared to those reported by Rominger, *et al.*, and by Von Haam and Beard might possibly be attributed to variations in the goat's milk or/and in the condition of the rats used. For example, the above observers used rats that weighed about 20-30 gm.

at the beginning of the experiments, whereas those used by György and by us averaged about 10 gm. heavier. Further experimental and clinical data are necessary before the specific character of a goat's milk anemia can be established.

Conclusions. 1. Exclusive feedings of goat's milk to young white rats produced an anemia that was not significantly different from the type of anemia produced with cow's milk. 2. The anemia from goat's milk showed maximal responses to iron and copper therapy.

8245 C

Phagological Identification of Streptococcus epidemicus.

ALICE C. EVANS.

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Davis and Rosenow[1] described the streptococcus which caused the Chicago epidemic of septic sore throat of 1911-12. They considered the disease to be a definite clinical entity like the Boston epidemic of 1911. The latter epidemic was described by Winslow,[2] who regarded it as the first of its kind to be described in this country. It was distinguishable from the throat disease which resembles atypical scarlet fever and which may be associated with definite scarlet fever cases.

Davis[3] gave the streptococcus of the Chicago epidemic the specific name *epidemicus*. Only 2 characters have been mentioned by investigators of this organism by which it may be differentiated from other hemolytic streptococci of human origin—the appearance of a capsule in young, moist cultures and the marked moisture and rapid drying of the colonies. Rosenow and others who studied *Streptococcus epidemicus* soon recognized the indefiniteness of capsule formation and moistness of colonies as specific characters. Rosenow reported that on artificial cultivation the epidemic sore throat strains assumed the characters of *S. pyogenes;* and that when cultivated in unheated milk *S. pyogenes* became modified so as to correspond with the streptococcus of epidemic sore throat. Ward and Lyons[4] and other investigators cited by them have recently shown that capsule formation and mucoid growth are characters of one of the variants of the hemolytic streptococcus of which another variant may occur with these characters lacking.

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[1] Davis, David J., and Rosenow, E. C., *J. A. M. A.*, 1912, **58**, 773.

[2] Winslow, C.-E. A., *J. Inf. Dis.*, 1912, **10**, 73.

[3] Davis, David J., *J. A. M. A.*, 1912, **58**, 1283.

[4] Ward, Hugh K., and Lyons, Champ., *J. Exp. Med.*, 1935, **61**, 515.

In studying a large collection of strains of hemolytic streptococci from a great variety of human and animal disease sources, the writer found that phagologically there is a distinction between a small group which is made up largely of septic sore throat strains and the large group which contains the majority of the scarlet fever strains of the collection. Fortunately, a strain from the Chicago epidemic was available for study. It fell into the small group, which thereby receives the name *epidemicus,* because the Chicago strain was the type strain of the species so designated by Davis.

The specific phagological characters of *S. epidemicus* will be considered here, leaving for a later publication the detailed description of other characters of the organism, its distribution, and the various kinds of infections which it may produce.

Sensitivity to nascent phage as a character for the differentiation of the beta type of streptococci was discussed in a previous publication,[5] and in another paper[6] a description was given of the 4 races of phage used in the classification studies. The histories of the streptococci used as substrata were also given in the earlier paper. The races of phage are designated A, B, C, and D. A lytic filtrate is designated by a letter for the race of phage and a number which designates the strain of streptococcus which served as a substratum, as, for example, A/751.

Every strain of streptococcus in the collection was tested for sensitivity to phages A/751, B/563, C/594, C/646, and D/693, in the nascent state. Two strains of phage C were used because it was found that the streptococcus serving as the substratum for the development of a phage influences the range of its activity, and the difference in the range of activity of C/594 and C/646 was found to be useful for classification purposes.

The stock supply of lytic filtrates was prepared as follows: Tubes containing about 10 cc. of broth were each planted with one drop of sensitive culture and one drop of lytic filtrate of the phage to be prepared. After incubation over night the contents of the tubes in which clearing occurred were filtered together through a Berkefeld N filter, tubed, and stored in the refrigerator for use.

The test for sensitivity of a streptococcus strain to one of the races of phage in the nascent state can be most clearly described by an example. In testing Streptococcus 599 for sensitivity to phage A/751, a drop of diluted filtrate A/751 and a drop of culture 751 are added to each of 2 tubes of broth. One of these tubes serves for the phage control. A control tube of broth is also planted with a drop of culture 751 alone. Then one of the tubes containing the diluted phage and culture, and a tube of sterile broth, are each planted with a drop of culture 599. All the tubes are incubated over night and examined the next morning. If the broth cultures 751 and 599 are turbid and the phage control is clear, readings are recorded. If the tube containing phage and planted with culture 599 is clear, or if it is definitely less turbid than the broth control, the reaction is recorded as positive; if the culture of 599 plus phage and the broth control culture 599 are equally turbid the reaction is recorded as negative.

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[5] Evans, Alice C., *Pub. Health Rep.*, 1934, **49**, 1386.

[6] Evans, Alice C., *J. Bact.*, 1935, **29**, 40.

The test for sensitivity to the other races of phage follows the same technic except that other strains of streptococci serve as substrata.

Streptococci were available for study from 21 epidemics of sore throat. The writer is indebted to many investigators for these strains. The collection contains more than one strain from many of the epidemics, but only one representative strain is considered in these data, except in the cases of the Boston 1917, Lee and Walton epidemics, from which the strains fell into 2 groups, and strains representing both groups are included. Strains from sporadic cases of tonsilitis or sore throat are not included in these data.

According to fermentation reactions 2 sore throat strains—the Barraboo, Wisconsin, 1929 strain and Griffith's[7] strain "Beatty" (Type 17)—were differentiated from the others by their fermentation reactions. They will not be further considered here. The remaining 22 strains were alike in their fermentation reactions,

TABLE I.
Phagological Characters of the Streptococci of Scarlet Fever and of Septic Sore Throat.

Group	Sensitivity to phage					No. of scarlet fever strains	% of scarlet fever strains	No. of sore throat strains	% of sore throat strains
	A/751	B/563	C/594	C/646	D/693				
I	+	+	+	+	—	66	58.9	4	16.6
II	+	+	+	—	—	1	0.9	2	8.3
III	+	+	—	+	—	12	10.7	8	33.3
IV (*Epidemicus*)	+	+	—	—	—	2	1.8	8	33.3
Total						81	72.3	22	91.5

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[7] Griffith, F., *J. Hyg.*, 1935, **34**, 542.

agreeing with the majority of the 112 scarlet fever strains of the collection in fermenting lactose, salicin and trehalose, but not mannite nor sorbitol.

The phagological reactions of the 22 sore throat strains are given in Table I. For comparison, there are also included in the table 81 scarlet fever strains which were indistinguishable from the sore throat strains. There are in the collection 31 scarlet fever strains not included in the table because they differed from those of the 4 groups either phagologically or in fermentation reactions.

The groups are designated by Roman numerals in the table, leaving for a later communication the discussion of what the names of the first 3 groups should be.

The septic sore throat strains were distributed among the 4 groups as follows:

Group I. Canton, Mass., 1913; Lee, Mass., 1928; Pittsfield, Mass., 1928 (Lee and Pittsfield are about 10 miles apart); and Griffith's strain "Symons" (Type 9).

Group II. Clyde, N. Y., 1930; Waterloo, N. Y., 1931 (Clyde and Waterloo are about 15 miles apart).

Group III. Boston, 1911; Boston, 1917; Plymouth, Mass., 1928; Kingston, N. Y., 1930; Kirkland Lake, Canada, 1931; Walton, N. Y., 1931; Topsfield, Mass., 1932; and Griffith's strain "Carter" (Type 13).

Group IV. Chicago, 1912; Boston, 1917; Saugerties, N. Y., 1925; Lee, Mass., 1928; Savannah, N. Y., 1929; Wayland, N. Y., 1929; Millbrook, N. Y., 1930; Walton, N. Y., 1931.

Whereas the majority of scarlet fever strains fell into Group I, only 16.6% of sore throat strains fell into that group. On the other hand, the majority of sore throat strains fell into Groups III and IV, which differ from Group I in resistance to phage C, either completely, as indicated by resistance to C/594 and C/646 (Group IV), or partially, as indicated by resistance to C/594 but not to C/646 (Group III). Group II is a small intermediary group. The *epidemicus* type strain fell into Group IV.

Only 2, or 1.8% of scarlet fever strains agreed with *S. epidemicus.* One of these strains was received from Budapest, with no history other than that it came from a case of scarlet fever. The other is Hooker and Follensby's[8] scarlet fever strain "Cook" which they found to produce "B" toxin almost exclusively.

Summary. Streptococcus epidemicus may be distinguished from the streptococcus which causes the majority of cases of scarlet fever by its lack of sensitivity to phage C. *S. epidemicus* rarely causes scarlet fever.

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[8] Hooker, Sanford B., and Follensby, Edna M., *J. Immunol.*, 1934, **27**, 177.

8246 P

Cytological Changes in Frog Pituitary Considered in Reference to Sexual Periodicity.

PAUL A. ZAHL. (Introduced by G. H. Parker.)

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Recent experiments with Amphibia indicate that the anterior lobe of the pituitary exerts a controlling influence not only on the development of the primary sex organs, but also on the release and discharge of the sex products. Wolf[1] and Rugh,[2] working with various Anura, have demonstrated that amplexus and shedding of the sex products can be induced in mid-winter by simple hetero- or homoplastic implantation of anterior lobe tissue. Houssay and Lascano-Gonzales[3] observed a degeneration of the testes following hypophysectomy in the toad. The writer wishes to present briefly the results of a cytological study of the frog anterior lobe, and on the basis of cytological evidence to postulate the rôle played by the pituitary in the mechanism of sexual periodicity.

Several species of the genus *Rana* were used in this study. At regular intervals throughout this annual cycle specimens were killed and the cytological condition of the anterior lobe pituitary was noted. Various sublimate and osmic acid fixatives were used in preparing the tissue for microscopic examination. Staining procedure most consistently used was a modification of the acid fuchsin-methyl green technique.

The general pattern of the winter frog anterior lobe is similar to that found in the other vertebrates. The numerical proportion, the inclusions and the structure of the cell types, however, do not conform entirely to those of the higher vertebrates, particularly mammals. The basophiles are small cells, few in number, and their general appearance does not suggest high secretory activity. True acidophiles, on the other hand, are larger and are filled with densely packed acidophilic granules. True chromophobes are as large as the acidophiles, but are more or less free of specific granulation. Grading between the true acidophilic type and the true chromophobic type are found cells exhibiting intermediate stages of acidophilic granulation, some containing comparatively few acidophilic granules, while others approach the true acidophiles in amount of granulation present. The size of the acidophilic particles varies from exceedingly fine granules to large coarse droplets of globules which have a distinct affinity for fuchsin and iron-haematoxylin. This size gradient suggests the origin of the latter from the former. The large fuchsinophilic droplets may occur in acidophiles as well as in the otherwise chromophobic cells.

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[1] Wolf, O. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 692.

[2] Rugh, Roberts, *Biol. Bull.*, 1934, **66**, 22; *Biol. Bull.*, 1935, **68**, 74.

[3] Houssay, B. A., and Lascano-Gonzales, J. M., *Compt. rend. Soc. Biol.*, 1929, **101**, 938.

A preliminary study of the seasonal and local distribution of the acidophilic inclusions reveals the following information. During the late summer and autumn months the anterior lobe exhibits a relatively low number of acid cells and a paucity of fuchsinophilic droplets. Throughout the winter months, while the frog is in a low metabolic condition, there is a numerical increase of true acidophiles, apparently at the expense of the chromophobes. The fuchsinophilic droplets in both cell types also increase in size and number. A quantitative maximum in this direction is attained in early spring. At this time the cytological appearance of the anterior lobe is predominantly acidophilic, and the fuchsinophilic droplets are large and present in almost all but the inconspicuous basophilic cells. The larger droplets are frequently found in the otherwise chromophobic cells. In the spring and summer immediately following discharge of the gametes there is a paucity of the large fuchsinophilic droplets, and the number of acidophilic cells is reduced.

These observations lend themselves to but one interpretation. The frog anterior lobe is active continuously throughout the year. During the summer months the secretion probably stimulates the development of the sex glands. By the end of summer, when the sex elements are completely developed, either the secretion can no longer be utilized or the sex glands inhibit the release of the hormone, and a storage occurs. This storage continues throughout the winter, and is evidenced by a progressive increase in acidophilic granules and fuchsinophilic droplets. During the breeding season the stored material is discharged from the pituitary. Its immediate effect on the organism is to induce amplexus and to cause a discharge of the mature sex products.

The Amphibia exhibit a great variety of breeding habits. A physiological mechanism, therefore, which is found to operate in one genus cannot be applied broadly to the entire group or even to other genera.

This cytological study of the pituitary in relation to sex activity is being extended into other groups of Amphibia. A more quantitative analysis of the work together with the results of castration and injection experiments will be published *in extenso* at a later date.

8247 C

Effect of Iron on Hemoglobin Regeneration in Gastrectomized Dogs.

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We have previously shown[1] that the gastrectomized dog has a markedly reduced capacity to regenerate hemoglobin when maintained at anemic levels on various diets. The average production capacity is approximately 10% of that of normal dogs and is around 0.2 gm. of hemoglobin per day on a meat diet. It was also noted that liver feeding or the administration of liver extract 343 (Lilly) is comparatively ineffectual in increasing the level of hemoglobin production. Liver extract 55 (Lilly), however, caused a definite increase in hemoglobin production. As this preparation contains added iron, the present studies were directed to determining the effect of the oral administration of iron. Incidentally, the character of the blood findings during the period of anemia and of iron therapy were followed.

The same 4 dogs used in the previous study were used, the gastrectomy operation thus preceding the present experiments by from 2 to 3 years. The technique of study was similar to that used previously. The dogs were maintained at an anemic level (*e. g.,* hemoglobin levels of 6 to 9 gm. per 100 cc.) by intermittent bleeding, the hemoglobin production for a given period being determined as the total hemoglobin removed by the bleedings plus or minus the gain or deficit in total circulating hemoglobin during the same time. This is the method developed by Whipple and his coworkers. The hemoglobin determinations were made with a Sahli-Hellige Haemometer and the blood volume determinations by the method of Keith and Rowntree as modified by Whipple. One dog (No. 12) was spontaneously anemic and was not subjected to bleedings. After several consecutive periods of standardization on a meat diet alone, iron in the form of Blaud's pills (Pills of Ferrous Carbonate), ferric ammonium citrate, or saccharated iron oxide was administered orally and the hemoglobin production during the iron regime determined. The essential data are shown in Table I. It is interesting to note that after a period of 2 years or more there is no compensatory improvement in hemoglobin production capacity on a meat diet. The data obtained previously are included in this table for this comparison. When iron is administered there is a definite and fairly marked increase in hemoglobin production. This is significantly less than that seen with normal dogs, however, as reported by Whipple, but shows that the gastrectomized dog, although definitely handicapped can nevertheless utilize added iron given orally. It is also

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[1] Mullenix, R. B., Dragstedt, C. A., and Bradley, J. D., *Am. J. Physiol.*, 1933, **105**, 443.

TABLE I.
Effect of Iron on Hemoglobin Production in Gastrectomized Dogs.

Dog No.		Hemoglobin Production gm. per day		Iron gm. Daily	Form of Iron Administration
		Days	Hb. gm. per day		
10	Meat diet—2 yr. prev.	29	0.00		
	” ” ” ”	24	0.33		
	” ” ” ”	37	0.236		
	” ” pres. exp.	31	0.00		
	” ” ” ”	32	0.25		
	” ” ” ”	38	0.00		
	” + daily iron	23	1.47	1.19	Fe. Am. Citrate
	” ” ”	46	0.91	.221	” ” ”
11	Meat diet—2 yr. prev.	30	0.127		
	” ” ” ”	37	0.298		
	” ” pres. exp.	52	0.144		
	” ” ” ”	63	0.243		
	” + daily iron	23	1.41	.560	Sacch. Iron Oxide
	” ” ”	46	1.63	.221	Fe. Am. Citrate
12	Meat diet—2 yr. prev.	54	0.00		
	” ” ” ”	37	0.373		
	” ” pres. exp.	30	0.00		
	(Spontaneously anemic)				
	Meat + iron	29	0.4	.3	Blaud's Pills
	” ”	25	0.74	.560	Sacch. Iron Oxide
	” ”	46	1.15	.221	Fe. Am. Citrate
14	Meat diet—2 yr. prev.	112	0.371		
	” ” pres. exp.	111	0.444		
	” + iron	46	0.9	.221	Fe. Am. Citrate
	” ”	27	2.13	.85	” ” ”

apparent that all of the increased hemoglobin production previously observed with liver extract 55 can be accounted for by the effect of the administration of iron.

Some of the data illustrating the changes in the blood picture of these dogs from the pre-anemic state to the anemia before and after iron administration are indicated in Table II. As previously reported, gastrectomized dogs even after periods of induced anemia do not develop a blood picture similar to that of pernicious anemia. The erythrocyte count remains practically normal with a marked microcytosis, low color index and low volume index. Upon the administration of iron, the color index increases, the volume index may increase slightly, and the saturation index increases. The spontaneous anemia occurring in one animal was also characterized by a marked microcytosis. Both the spontaneous and induced anemia in the gastrectomized dog is therefore a microcytic anemia, relatively refractory to liver, but responding well to iron therapy.

TABLE II.

Blood Findings—Gastrectomized Dogs During Induced Anemia With and Without Iron.

	R.B.C. mil. per mm^3	Hb. gm. per 100 cc.	Cell Vol. %	Mean Corp. Vol. μ^3	Mean Corp. Hb. gg*	Mean Corp. Hb. conc. %	Color Index	Volume Index	Saturation Index
10.									
Pre-anemic status	7.15	15.0	48	67	21	31	.95	1.03	.92
90 days anemic	7.2	8.9	29	40	12	30	.55	.61	.90
23 ‘‘ on iron	7.23	11.7	29	40	16	40	.73	.61	1.19
11.									
Pre-anemic status	7.15	13.6	50	70	19	27	.86	1.07	.80
115 days anemic	5.8	8.1	18.5	32	14	43	.62	.49	.96
23 ’’ on iron	6.91	10.7	25.6	36	15	41	.70	.56	1.26
12.									
Spontaneous anemia	5.5	4.0	13	23.6	7	30	.33	.37	.9
101 days on iron	7.2	8.0	18	25	11	44	.5	.38	1.3
14.									
Pre-anemic status	6.69	14	45.8	68	21	30	.95	1.04	.91
111 days anemic	6.0	6.9	20	33	11.5	34	.52	.46	1.0
27 ’’ on iron	6.35	12.9	34	52	20	38	.92	.82	1.12

Note: Cell volume % determined—10 cc. blood with 2 cc. 1.6% sodium oxalate—centrifuged 30 min. 2500 R.P.M. For calculation of Indices. 16 gm. Hb. per 100 cc. = 100%, 7.2 R.B.C. per cu. mm. = 100%, cell volume 47 per cent = 100%.

*Micromicrograms.

8248 P

Effect of Sublethal Doses of Monochromatic Ultraviolet Radiation on Bacteria in Liquid Suspensions.

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From the Laboratory of Plant Physiology, University of Wisconsin.

In the course of a quantitative investigation[1] of the lethal effect of monochromatic ultraviolet radiation below 3,000 A on stirred saline suspensions of bacteria *(Escherichia coli)* by a method similar to one described in a previous publication,[2] we observed the following phenomena:

If the bacteria are plated out in nutrient agar on Petri dishes subsequent to radiation, the colonies formed by the bacteria which survived the irradiation appear later than the colonies formed by the bacteria from the control suspension treated in the identical manner but protected from the ultraviolet radiation.

A careful study of the growth of the irradiated and control suspensions in a relatively poor medium (3 gm. NaCl, 0.2 gm. KCl, 0.2 gm. $CaCl_2$, 0.04 gm. Difco beef broth powder, 1,000 cc. distilled water, ca 10,000 bacteria/cc.) was made by determining the number of viable, colony-forming, organisms at proper intervals and several interesting observations were made:

1. The retarded growth (lag) phase of the irradiated bacteria was extended considerably over the control. This extension apparently depends on the energy applied to the suspension. When the bacteria had completed their growth, the suspension with the control and that with the irradiated bacteria contained the same number of organisms.

2. A careful determination of the lag phase revealed that where the control culture changed little in number of bacteria during this phase, the culture which had survived irradiation increased in number quite rapidly in the earlier part of the lag phase and then slowed down more or less for a certain time before it came into the log phase, thus producing a modified extension of the lag phase. The growth curve of the control rising in the beginning of the lag phase

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* Under the auspices of the Radiation Committee of the Division of Biology and Agriculture, National Research Council.

[1] Hollaender and Claus, forthcoming publication.

[2] Duggar, B. M., and Hollaender, Alexander, *J. Bact.*, 1934, **27**, 219, 241.

actually crossed the curve of the exposed culture and in the end an extension of the lag phase was induced by the radiation. The total number of viable bacteria of the exposed culture had increased during the lag phase. These increases were so pronounced that in the time during which the control culture showed a change in number of not more than 10 to 15%, the exposed culture had increased up to, or more than, 100%.

Tests have shown that the increased growth in the lag phase of the exposed culture was not produced by the decomposition products of the dead bacteria or by the excretion of any substance by the irradiated organisms. Stimulation is suggested, but the possibility of recovery of the irradiated bacteria is not entirely excluded.

The observed phenomena do not refer to the effects of very small amounts of energy, but only to energy values which kill some of the bacteria and allow the rest of the organisms to survive.

A detailed description of the experiments will be published later. The authors wish to thank Prof. B. M. Duggar for the help and advice he has given them in the course of this investigation.

8249 P

Effect of Infrared on Tissue Temperature Gradient as Influenced by Pigment.

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Determinations of the temperature gradients[1] from the surface of the skin to a depth of 16 mm. in 7 subjects with skin pigment types ranging from blond to negro, before, during and after exposure to varying quantities and wave bands of infrared radiation, indicate that the rise in temperature on the surface is proportional to the quantity of radiant energy and independent of wave length, between 0.75μ and longer than 5.0μ, and the amount of natural skin pigment.[2] The wave lengths of infrared used were broad bands 0.75μ to 3.0μ, 1.4μ to 5.0μ, 1.4μ to longer than 5.0μ, 3.0μ to longer than

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[1] Temperature gradients determined by a method similar to that of Bazett, H. C., and McGlone, B., *Am. J. Physiol.*, 1927, **82**, 415.

[2] Our present knowledge of the function of skin pigment is reviewed by Laurens, H., 1933, The Physiological Effects of Radiant Energy. Chemical Catalog Co., New York, p. 122.

5.0μ and the quantities varied from 0.25 to 1.6 gm. cal. per sq. cm. per min.[3]

That there is no relation between the degree of natural pigmentation and the tissue temperatures during infrared irradiation is illustrated by experiments summarized in Table I, which shows tissue temperatures after 5-minute exposures to 1.0 gm. cal. per sq. cm. per min. of infrared (wave length 1.4 to longer than 5.0μ). The differences between these various skin types are no greater than those in a single individual on different days. Other wave bands gave similar results.

TABLE I.

Depth	Negro	Blond White	Japanese	Brunet White
Surface	44° C	45.0° C	44.0° C	43.5° C
2 mm.	40	40.5	40.0	39.0
6-8 mm.	38	38.0	38.0	37.4
15 mm.	37	36.5	36.8	36.8

On the other hand, when skin areas, artificially pigmented by exposure to a carbon arc, are exposed to the same amount of infrared they show somewhat smaller rises in temperature on the surface and at sub-surface levels than do corresponding non-pigmented areas. A typical experiment using 1.0 gm. cal. of long infrared (1.4μ to longer than 5.0μ) is summarized in Table II. We do not believe that the artificial pigment necessarily serves a protective function, but that an increased blood flow (note the higher initial temperatures in the pigmented skin) is responsible. This conception is more

TABLE II.

	Non-Pigmented Skin		Artificially Pigmented Skin	
Depth	Before	End of 5 min. irradiation	Before	End of 5 min. irradiation
Surface	32.5° C	46.5° C	34.0° C	43.2° C
2 mm.	33.3	42.2	34.9	38.6
7 mm.	34.5	38.0	35.1	37.5
16 mm.	35.5	36.7	35.6	36.6

TABLE III.

	Normal Skin		Erythematous Skin	
Depth	Before	End of 5 min. irradiation	Before	End of 5 min. irradiation
Surface	32.0° C	46.0° C	35.5° C	43.2° C
2 mm.	33.0	42.0	36.2	39.8
5-6 mm.	34.5	40.5	36.7	39.0
15 mm.	36.0	37.2	36.9	37.3

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[3] Physical methods used are described by Laurens, *op. cit.*, p. 16.

strikingly supported by experiments on markedly inflamed skin (produced with a carbon arc), a typical example, using 1.0 gm. cal. doses of long infrared (1.4μ to longer than 5.0μ) being summarized in Table III.

Other means of altering the rate of blood flow through skin influence the temperature changes due to irradiation. When the circulation of the leg is occluded by a tourniquet irradiation results in higher temperatures at all depths to 16 mm. than in a leg with normal circulation. On reestablishing the circulation of the leg after 30 or more minutes of occlusion, a reactive hyperemia occurs (indicated by higher than normal initial tissue temperatures) and then irradiation is found to result in temperature rises at all depths which are less than normally observed.

Conclusions. Pigment, natural or artificial, does not appear to protect deeper regions of the skin from excessive heating due to infrared. The slight diminution in heating in heavily artificially pigmented skin is probably due to increased blood flow.

8250 C

Hepatoflavin and Pernicious Anemia.

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The following reasons led to the idea that hepatoflavin might be related to the hematopoietic factor used in treating pernicious anemia. Liver and liver concentrates of therapeutic value are rich sources of flavin. Flavin appears to be one of the components of vitamin B_2, which has been regarded as concerned with the extrinsic factor of Castle. West and Howe[1] reported that the hematopoietic activity of their most potent liver concentrate was precipitated from alcoholic solution by $Ba(OH)_2$; we find that the flavin in liver extracts is similarly precipitated. It seemed desirable to test whether flavin might be effective in pernicious anemia therapy.

Incubation of liver with pig stomach, which appears to increase the hematopoietic activity, does not increase the flavin content.

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*National Research Council Fellow.

[1] West, R., and Howe, M., *J. Clin. Invest.*, 1930, **9**, 1.

Purified flavin prepared from liver by a process described elsewhere, has been administered to 5 patients. The material was given by intramuscular injection, in amounts (5 cc.) representing 500 gm. of fresh liver. The criteria of effect were the hemoglobin, erythrocyte, and reticulocyte response. The 5 patients studied in this manner gave uniformly negative results. Since no effect was noted it is not necessary to record the protocols. The following statement is representative. Five cc. of flavin solution were given on alternate days until a total of 25 cc. had been injected. No significant change in the blood picture was noted during this period. The patient was then given commercial liver concentrate, which was followed by the typical reticulocyte response, generally on the fourth day.

One patient was treated by oral administration of Lilly's liver extract "343" which had been irradiated for 24 hours with a 300 watt light bulb. The irradiation was done at 15° in 10% NaOH solution, a treatment proved to change the flavin into photoflavin. After irradiation the material was neutralized, evaporated, and extracted with 80% alcohol. The alcohol extract was evaporated and the residue fed to the patient. A positive response was obtained, showing that the photolysis of the flavin had not impaired the hematopoietic activity of the material.

From these observations it may be concluded that liver flavin, given intramuscularly to patients with pernicious anemia does not exert an hematopoietic response; and that liver extract, the flavin of which has been converted to photoflavin, is still active.

8251 C

Influence of Estrogenic Substance upon Experimental Syphilis of the Adult Male Rabbit.

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It is well known that the human female is more resistant to syphilitic infection than the male, especially during the child bearing period of life. This difference has been attributed to the influence of pregnancy.[1, 2] Corroborating the observations on human individ-

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[1] Moore, J. E., *Arch. Int. Med.*, 1922, **30**, 548.

[2] Moore, J. E., *Bull. Johns Hopkins Hospt.*, 1923, **34**, 89.

uals, there is experimental evidence that the pregnant rabbit inoculated at the time of conception reacts to infection less severely than either the normal female or male rabbit.[3] A similar sex variation has been reported in normal rabbits.[4]

The essential nature of the forces responsible for this immunologic dissimilarity of the sexes is not known. In seeking an explanation, it is reasonable to assume that some endocrine function peculiar to the female and closely related to reproduction may play a dominant rôle in the protective mechanism of this sex. In this connection it has been shown that the reaction of the rabbit to syphilis depends in part upon the integrity and balance of the glands of internal secretion.[5]

One of the most striking phenomena of pregnancy related to the activity of the glands of internal secretion is the elaboration of an estrogenic substance, or substances, which as pregnancy progresses can be demonstrated in large amounts in the circulating blood[6] and in the urine.[7] As it is known that certain secondary sex characters of the female depend upon the presence of this substance, it has occurred to us that the resistance of the female to syphilitic disease may be an analogous character of sex dependent upon the same or similar factors.

The following experiment was undertaken for the purpose of observing the effect of estrogenic substance upon the course of syphilitic infection in the adult male rabbit. The feminizing effects of the prolonged administration of this substance on the animals employed in this experiment have already been described.[8]

Estrogenic substance was prepared by extracting acidulated human pregnancy urine with butyl alcohol by the method described previously.[8] The extract was heated to a temperature of 100°C. for at least 12 hours during the process of extraction. After removal of the solvents the residue was taken up in olive oil and assayed for its estrogenic content. The quantity required to produce estrus in 50% of 20 ovariectomized, sexually mature, albino rats was the rat unit employed in the experiment.

Mature, male albino rabbits, 16-17 months of age, from a pure albino stock were used. The animals had been caged and fed from birth under uniform conditions, and had never been mated.

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[3] Brown, W. H., and Pearce, L., *Am. J. Syph.*, 1920, **4**, 593.

[4] Chesney, A. M., *J. Exp. Med.*, 1923, **38**, 627.

[5] Pearce, L., and Van Allen, C. M., *J. Exp. Med.*, 1926, **43**, 297.

[6] Frank, R. T., Frank, M-L., Gustavson, R. G., and Weyerts, W. W., *J. A. M. A.*, 1925, **85**, 510.

[7] Aschheim, S., *Arch. f. Gynäk.*, 1927, **132**, 179.

[8] Frazier, C. N., and Mu, J. W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 997.

The strain of *Treponema pallidum* employed was that originally isolated by Nichols. The immediate source of virus was the testicular tissue of a rabbit with a progressing syphilitic orchitis. On May 1, 1934, when the inoculations were made, the strain had undergone 31 serial animal passages since being brought to this laboratory in 1927.

From 20-60 rat units of estrogenic substance in olive oil were injected subcutaneously once a day, 6 days a week, into 8 rabbits. A second series of 8 rabbits were injected in like manner with a corresponding amount of plain olive oil. Injections were continued in each case for 250 days, or as long as the animal survived.

Fifteen days after the first injection, each animal of both series was inoculated with 0.3 cc. of a saline emulsion of testicular tissue containing from 2-3 actively motile organisms to the microscopic field. The 2 groups were inoculated alternately so that no 2 animals from the same group received material from the same syringe. All inoculations were made into the right testis.

A primary orchitis developed in each animal. The mean incubation period for the estrin group was 16.4 days, and for the olive oil group, 20.3 days. This difference is probably not significant. The primary testicular reaction of the 2 groups differed particularly with respect to its chronicity. The mean duration of orchitis in the former group was 58.4 days as compared with 141 days in the control group. Healing of testicular lesions in those animals treated with estrogenic substance coincided with enlargement of the nipples, the first manifestation of the physiologic activity of the estrogenic substance, and in all animals except one the period of primary orchitis was terminated with the establishment of lactation. Milk could be expressed from the mammary glands of these animals 80 days after the first injection of the estrogenic preparation, which was 65 days after inoculation with *T. pallidum.*

Scrotal chancres developed in 7 of the control rabbits and in 4 of the test rabbits. The scrotal lesions of the latter group originated as circumscribed foci in the scrotal skin and were not direct extensions of testicular lesions.

There was a significant difference in the incidence of metastatic orchitis between the 2 groups. Six control animals developed lesions of the uninoculated testis at a mean time of 69.5 days after inoculation, but not a single rabbit treated with estrogenic substance developed metastatic foci of the uninoculated testis, and in only 1 animal was there a transient localized infiltration of the head of the epididymis. The 2 rabbits in the control group without metastatic lesions of the testis, developed ulcerative reactions of the scrotum, and 2 test animals showed similar reactions.

An important criterion of the effectiveness of the resistance of the rabbit to syphilis is the character and extent of the generalized metastatic lesions. With respect to the location and number of these, the control animals had in all 16 detectable lesions as compared with 5 in the treated animals. The distribution of lesions was as follows: Control group: periosteum and bone 11, skin 3, and eyes 2. Test group: periosteum and bone 2, skin 0, and eyes 3. There was, moreover, a distinct delay in the appearance of generalized lesions among the animals treated with estrogenic substance. This was particularly true with respect to the periosteal and bone lesions. Among those animals developing reactions in the skeletal tissues, the difference between the 2 groups in the mean time of appearance of the lesions was 57 days, while the mean duration of such lesions in the control group was 28 days and in the test group, 14 days.

A further means of comparison is in the behavior of the 2 groups with respect to the number of cases in which resolution and healing of lesions was complete at the expiration of the experiment. Seven of the 8 animals injected with plain olive oil survived for 227 days. All of these had active lesions at that time, while only 2 of the 6 surviving animals treated with estrogenic substance presented signs of active infection.

A final comparison of the difference in susceptibility may be made on the basis of the actual number of days during which the animals were free from detectable manifestations of disease. For this summary only those rabbits surviving the full period of 227 days are included. In the control group (7 rabbits) there was a total of 105 days of latency and in the test group (6 rabbits), a total of 651 days. As the phenomenon of latency in syphilis may be taken as a measure of the effectiveness of the forces opposing the infection, this difference is especially significant and indicates that the feminized male rabbits possessed a more adequate degree of protection against infection than the normal male rabbits.

Conclusions. Under the conditions of the experiment, the estrogenic substance employed exerted an inhibitory influence upon a syphilitic infection when administration of the preparation was begun shortly before inoculation. The first evidence of this effect, in the form of resolution of the primary orchitis, coincided with the earliest manifest physiological action of the substance on the mammary glands. Further evidence of a similar nature was in the absence of metastatic orchitis, and in the delayed appearance and shorter duration of generalized lesions. The effectiveness of the defensive reaction was particularly well demonstrated by the predominance of latency in the treated animals. The degree of protection afforded the testis, which as a rule is highly vulnerable to syphilitic infection, was the most noteworthy feature of disease resistance in the feminized males.

8252 C

Cocaine Recovery from Rabbit and Cat Aqueous Humor.

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In 1930 the statement was made "At present there is no method sufficiently delicate to determine quantitatively the amount of cocaine present in the anterior chamber as it arrives from the conjunctival sac after clinical instillation."[1]

Recently, a method[2] was developed whereby cocaine can be quantitatively recovered from body fluids. The essential features of this method follow:

To the deproteinized fluid supposedly containing cocaine, is added 1 cc. of a 1% solution of phosphomolybdic acid in normal sulphuric acid. After mixing and centrifuging, the precipitate is washed with water which, after another centrifuging, is decanted and discarded. One cc. of N/5 sulphuric acid, 1 cc. of 2% aqueous solution of hydroquinone, and after shaking, 1 cc. of carbonate-sulphite solution are successively added to the precipitate. This develops a color which is read in the Duboscq microcolorimeter against a standard prepared by adding the above reagents to 1 cc. of a solution of approximately equal alkaloid content.

With solutions of cocaine in aqueous humor the probable analytical error is 20% or less with the concentrations employed, which concentrations are approximately at the limit of colorimetric reading.

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[1] Yonkman, F. F., *J. Pharm. and Exp. Therap.*, 1930, **40**, 195.

[2] Walker, B. S., and Walker, E. W., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 148.

By this method we have succeeded in recovering cocaine from rabbits' aqueous humor in so small a concentration as 1-18,000, and 1-25,000 in aqueous humor of the cat.

Normal rabbits and cats of both sexes and various breeds received, after pupillary measurements, several instillations of 4% cocaine until the cornea became desensitized and a pupillary dilatation of 2 mm. or more occurred under uniform lighting. This usually required from ½ to 1½ hours. The animals were then lightly anesthetized with ether; the eyes were washed with normal saline in most cases, and the aqueous humor was extracted with the hypodermic needle and tuberculin syringe by means of the sclero-corneal approach used by Koppanyi.[3] In some instances the same rabbits were used for similar experiments after 48 hours, since the aqueous humor extraction works no particular hardship on the eye if carefully done and followed by protargin wash.

Our data were obtained from 50 experiments on 25 animals. Of these, 24 yielded recoverable cocaine, 5 served as controls and 16 failed to show cocaine in the aqueous humor although respective pupils were noticeably dilated. Of the failures recorded, 5 appeared in 6 cat experiments, the remaining 11 appearing in the rabbit eyes. However, in 2 instances attempted, 0.12 mg. of cocaine was regained from 0.15 mg. added to the clear, control aqueous humor, suggesting extremely minute amounts of cocaine responsible for pupillary dilatation in some eyes.

Conclusions. The amount of cocaine recovered from anterior chambers of rabbit and cat eyes, whose pupils were dilated at least 2 mm. after conjunctival administration varied from 1-5,000 to 1-25,000. Beyond this dilution no recovery was encountered even though the pupils in these failures of recovery were dilated 2 mm. or more. We feel justified in stating that failure to recover cocaine in these instances can be accounted for on the basis of amounts of cocaine physiologically active but too small to be recoverable by this procedure. This is supported by experiments[2] in which known amounts of cocaine were added to control aqueous humor and recovered almost *in toto,* but not beyond the limits of 1-25,000. Further, our stock solution (4%) checked accurately against fresh cocaine standard, even after 3 and 4 weeks' usage.

These findings are of interest in view of the fact that in an earlier paper[1] on the physiological action of cocaine on isolated steer irides, the following conjecture was offered: " . . . it would seem that

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[3] Koppanyi, T., *J. Pharm. and Exp. Therap.*, 1930, **38**, 113.

1:10,000 or perhaps more safely, 1:15,000 or 1:20,000 concentration of cocaine more closely approximates the concentration found at the iris sufficient to produce dilatation."

At present, we are studying cocaine recovery following clinical application in man.

8253 P

Influence of Hormones on Agglutinin Response to B. Pertussis in Immature Rats.

JOHN H. DINGLE, ROLAND K. MEYER, AND EDWIN L. GUSTUS.

From the Department of Bacteriology and the Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

The inability of immature animals to respond to active immunization with the degree of demonstrable antibody production found in mature animals has been noted by numerous investigators.[1] Our experiments carried out in infantile, adolescent, and mature rabbits and rats indicated that increase in ability to form agglutinins to *B. pertussis* and *B. typhosus* was approximately coincidental with sexual maturation.

In an attempt to determine more accurately the effect of increasing age on agglutinin production and to correlate this, if possible, with some easily determined index of sexual development, the following experiment was performed. Ten groups of 5 male rats each were selected. The age of the groups increased by increments of 7 ± 1 days, the youngest group being 4 days old. All the rats were injected intraperitoneally with total doses of 9,000 million *B. pertussis* in 6 injections: 1,000 million per day for 3 days, 4 days' rest, and 2,000 million per day for 3 days. On the 17th day of the experiment, the animals were weighed and bled. Seminal vesicles and testes were weighed as an approximate measurement of the degree of sexual maturity. As the rats advanced in age, the results showed a general correlation between the rise in agglutinin titer on one hand, and the increase in testes, seminal vesicle, and body weights on the other. For example, the average body weight, seminal vesicle weight, testes weight, and agglutinin titer of 4-day-old rats were 34 gm., 8 mg., 182 mg., and 120, respectively; whereas those of 74-day-old rats were 211 gm., 511 mg., 2660 mg., and 2480.

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[1] See review of literature by Baumgartner, L., *Yale J. Biol. and Med.*, 1934, **6**, 403.

Since sexual development and growth are both influenced by the endocrine balance, it appeared possible that changes produced by administration of gonadotropic and estrogenic hormones to immature animals might be reflected in the response of these animals to active immunization.

Sixteen immature male rats (3-5 days old) were injected with highly purified gonadotropic hormone from pregnant mare serum,[2] 22 with extracts of human pregnancy urine,[3] and 5 male and 6 female rats with crystalline theelin. Adult male rat pituitary glands were implanted intramuscularly in 18 immature male rats. Thirty-one male rats of the same age served as controls. All animals received a total intraperitoneal dose of 9,000 million *B. pertussis* as outlined above.

Administration of the hormones produced marked response as indicated by increase in size of the testes and seminal vesicles, and in the case of the female by increase in size of the uterus and an oestrous vaginal smear. Implantation of pituitary glands produced little if any increase in the size of testes or seminal vesicles.

The average agglutinin titers of the above groups were as follows: gonadotropic hormone from pregnant mare serum, 19; prolan, 19; theelin, 25; pituitary implants, 54; and normal controls, 36. As can be seen from this data, no significant difference existed between the agglutinin titers of normal immature rats and those receiving gonadotropic and estrogenic hormones.

8254 C

Activation of Partially Purified Pepsinogen.

HEINZ HOLTER* AND JOHN H. NORTHROP.

From the Laboratories of the Rockefeller Institute for Medical Research, Princeton, N. J.

Pepsinogen can be extracted from the mucosa of the fundus part of pig stomachs by m/10 $NaHCO_3$-solution. Such extracts contain 80-90% of the total peptic activity of the mucosa (as determined after complete autolysis of the mucosa at pH 2) in the form of pepsinogen which is stable at alkaline reaction. The extract is treated with Filtercel† to remove most of the mucilaginous material, which otherwise renders the extract very difficult to handle. The pepsinogen may then be gradually purified by fractional precipitation with ammonium sulphate at pH 7. (Table I.)

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[2] Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

[3] Katzman, P. A., and Doisy, E. A., *J. Biol. Chem.*, 1934, **107**, 513.

* Fellow of the Rockefeller Foundation.

TABLE I.

Preparation	Yield		Q =
	gm.*	peptic activity $[P.U.]^{Hb}$	$[P.U.]^{Hb}_{mg}$ Prot. N
3000 ml $NaHCO_3$ extract from 830 gm. mucosa treated with 10% (weight) Filtercel, filtered with suction, washed with m/10 $NaHCO_3$, united filtrates (2900 ml.) 0.7 saturated with $(NH_4)_2SO_4$, after 12 hr. at 5° filtered with suction, filtrate discarded	110 g	170	0.045
Ppte. dissolved in m/10 Phosphate buffer pH 8, fractionated between 0.4 and 0.7 sat. $(NH_4)_2SO_4$ (final volume at 0.4 sat. = 1900 ml, final pH = 7.2)	—	—	0.079
Fractionation repeated between 0.46 and 0.7 sat., other conditions unchanged	35 g	90	0.105

Further fractionation was tried by means of ammonium sulphate at pH 5; this yields between 0.35 and 0.45 sat. products with Q as high as 0.145. These, however, proved to be partly activated and are therefore not discussed here. Similar results were obtained by fractionation with $MgSO_4$ at pH 5.

* Weight of moist filtercake.

The total peptic activity (pepsinogen + preformed pepsin) of the various samples was determined by the hemoglobin method[1] at pH 1.8, all pepsinogen being activated at this pH.[2, 3] Pepsinogen alone was estimated as the difference between the total activity just mentioned and the activity of samples which previously had been titrated to pH 9 in order to destroy preformed pepsin. The activity is expressed in pepsin units $[P.U.]^{Hb}$,[4] the "specific activity" (Q) being the ratio between the total number of pepsin units (pepsinogen + pepsin) and the amount of nitrogen precipitated by 4% CCl_3COOH at 80°: $\frac{[P.U.]^{Hb}_{ml}}{\text{mg Protein-N/ml}}$. The course of one preparation is given schematically in Table I.

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† A commercial preparation of infusorial earth.

[1] Anson, M. L., and Mirsky, A. E., *J. Gen. Physiol.*, 1932, **16**, 59.

[2] Ege, R., and Menck-Thygesen, P., *Biochem. Z.*, 1933, **264**, 13.

[3] Kleiner, I. S., and Tauber, H., *Z. physiol. Chemie*, 1933, **220**, 205; *J. Biol. Chem.*, 1934, **106**, 501.

[4] Northrop, J. H., *J. Gen. Physiol.*, 1932, **16**, 41.

Preparations with Q = 0.1 are but slightly colored, not mucilaginous, readily soluble in salt solutions at pH >5, stable between pH 6 and 9.

Attempts to crystallize pepsinogen have been unsuccessful so far, due either to impurities or to the fact that the isoelectric pH range, usually most suitable for the crystallization of proteins, is difficult to approach. Judging from the solubility in $(NH_4)_2SO_4$-solutions, the isoelectric pH seems to be <4 and at this pH the rate of activity is considerable.

The preparations Q = 0.1 undoubtedly contain some impurity, but in view of the fact that Q of crystalline pepsin previously has been found to be 0.2[4] and that part of the protein nitrogen of pepsinogen is split off during activation, we do not think that the amount of inert proteins present could be very high.

The rate of activation at pH 4 corresponds with the values found by Ege and Menck-Thygesen[2] in crude extracts and with a different technique. During activation there is an increase in the amount of nitrogen not precipitated by 4% CCl_3COOH at 80°. As shown in Table II, this increase runs parallel with the activation and stops at the same time. Up to this point the pepsin content increases, while the pepsinogen + pepsin remains constant. As a result Q increases and at the end of the reaction has risen to 0.2, the value for

TABLE II.

Preparation Q = 0.09, Pepsin + Pepsinogen = 0.057 $[P.U.]^{Hb}_{ml}$; pH = 4 (Acetate buffer); 35.5°.

Time	mg Prot. N/ml	Pepsinogen $[P.U.]^{Hb}_{ml}$	Pepsin $[P.U.]^{Hb}_{ml}$	% activated	% of N split off	Q
0	0.62	0.057	0	0	0	0.092
50 sec.	0.60	0.052	—	9	6	0.095
5 min.	0.53	0.042	—	26	26	0.11
13 ”	0.39	0.016	—	72	66	0.15
23 ”	0.30	0.003	—	95	92	0.19
60 ”	0.29	0.002	—	97	95	0.20
210 ”	0.27	0.001	0.056	98	100	0.21

Preparation Q = 0.15, Pepsin + Pepsinogen = 0.068 $[P.U.]^{Hb}_{ml}$ (About 70% of Pepsinogen activated during preparation); pH = 4 (Acetate buffer) 35.5°.

0	0.44	0.020	0.048			0.15
40 sec.	0.43	0.018	—			0.16
2 min.	0.40	0.014	—			0.17
5 ”	0.38	0.009	—			0.18
10 ”	0.33	0.005	—			0.21
19 ”	0.32	0.004	—			0.21
25 ”	0.32	0.003	0.065			0.21

crystalline pepsin. This seems to indicate that during activation some part of the pepsinogen molecule is split off, thus transforming it into pepsin.

How this cleavage is performed, however, remains for the time being unexplained. It will be noted that the activation rate is practically constant throughout the whole reaction, in agreement with the results of Ege and Menck-Thygesen.[2] This is difficult to explain and we do not feel prepared for any discussion of the mechanism of the activation until further experimental evidence has been obtained. We should like to mention, however, that there is some slight evidence in favor of the assumption that the cleavage might be a proteolytic reaction (slow activation after infection with bacteria, *Cf.* Ege and Lundsteen,[5] Kleiner and Tauber[3]—we have had similar experiences), and we have tried, therefore, to activate pepsinogen by trypsin and papain. These attempts have been unsuccessful.

8255 C

Avitaminosis. XVII. Influence of High Fat-Containing Diets on Vitamin B_1 Requirements.*

BARNETT SURE AND K. S. BUCHANAN.

From the Department of Agricultural Chemistry, University of Arkansas.

In a previous investigation[1] it was demonstrated that as much as 30% of fat in the diet has no sparing action on vitamin B requirements for lactation of the rat. In this study the problem has been extended to the non-lactating rat.

The following series of experiments were planned with the objective of increasing the protein as well as the fat content of the ration, at the same time keeping constant a high vitamin G content by the introduction of 15% of dehydrated autoclaved beef. The beef, purchased as round steak, was freed from all visible fat and bones and after autoclaving and drying at 100°C., contained 83% crude protein. The composition of the rations is given in Table I. It will be noted that the protein content varied from 19.98% to 33.37%, and the fat content ranged from 0 to 50%. That all the rations had a high vitamin G content became apparent after excellent growth was secured by the daily supplementation of a vitamin B concentrate, which was a Fuller's earth adsorbate kindly furnished by Dr. Jansen of Holland. Food consumption records showed that there was a daily intake of 500 to 600 mg. of autoclaved beef, which should have assured an abundant intake of vitamin G.

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[5] Ege, R., and Lundsteen, E., *Biochem. Z.*, 1934, **268**, 164.

*Research paper No. 380, Journal Series, University of Arkansas, Fayetteville.

[1] Sure, B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 622.

The results of 5 groups of animals on the dietary regime outlined in Table I disclosed that neither increasing the fat nor the protein in the ration, containing an abundance of vitamin G, is followed by any noteworthy sparing action on vitamin B_1 requirements of the non-lactating albino rat.

TABLE I.
Composition of Rations.

	A	B	C	D
Casein†	9	12	19	25
Salts No. 185	4	4	4	4
Autoclaved Beef	15	15	15	15
Lard	0	10	30	50
Dextrin	72	59	32	6
Protein Content (N × 6.25)	19.98	22.27	28.35	33.37

† Purified according to method described in PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 779.

Our next efforts were to determine if administering sub-optimum daily allowances of vitamin B_1, additional increment of growth would become apparent on rations containing greater proportions of protein and fat. Numerous experiments, the rations of which were supplemented with increasing amounts of dehydrated baker's yeast, Sure's vitamin B concentrate, and Jansen's Fuller's earth adsorbate, showed negative data.

From the character of our results, therefore, we conclude that. under the dietary regime we employed, high fat diets have no sparing action on vitamin B_1 requirements, even in the presence of an abundance of protein and vitamin G.

Our findings are in accord with the general conclusions in this field of study with the recent results of Steenbock and Kemmerer[2] but do not concur with the recent results of Evans and Lepkowsky.[3]

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[2] Steenbock, H., and Kemmerer, A. R., Bulletin 428, Wis. Exp. Sta. Report, 27.

[3] Evans, H. M., and Lepkowsky, S., *J. Biol. Chem.*, 1934, **107**, 429.

8256 P

Vitamin B_1 and Thyroxin.*

BARNETT SURE AND K. S. BUCHANAN.

From the Department of Agricultural Chemistry, University of Arkansas.

Sure and Smith[1] reported on the protective action of vitamin B against the toxicity of thyroxin administered subcutaneously or orally to the albino rat.

In this investigation attempts were made to balance quantitatively the toxic influence of thyroxin given orally to the rat with a much more concentrated vitamin B_1 extract; also with Williams' crystalline vitamin B_1 (manufactured by Merck).

The vitamin B_1 concentrate was furnished by the Eli Lilly Research Laboratories and is a product that is being tested clinically. This preparation is indicated for parenteral use, each cc. containing 150 Sherman units of vitamin B_1.

Seventy sets of animals in groups of 4 were used for this study, which were litter mates of the same sex. In each set, animal A received a diet deficient only in vitamin B_1, which represented the negative control; animal B received the diet of animal A, and in addition a daily dose of thyroxin; animal C received the same diet, a daily dose of thyroxin, and a daily dose of either the Lilly concentrate or crystalline vitamin B_1; animal D received the same diet, a daily dose of either source of vitamin B_1, but no thyroxin, which represented the positive control. The thyroxin was given daily in doses ranging from 0.05 mg. to 0.2 mg. The Lilly vitamin B_1 concentrate was furnished in doses supplying 1.5 to 30 Sherman units daily. The crystalline vitamin B_1 was administered daily in amounts ranging from 1γ to 300γ or 0.001 mg. to 0.3 mg.

The criterion of complete protection against thyroxin poisoning was not only the ability of the animal to survive but also to make as good a growth as if thyroxin were not introduced as a supplement to the experimental diet.[2]

Lilly Vitamin B_1 Concentrate. The best protection (75 to 100%) was obtained on a daily dose of 0.05 mg. thyroxin. This was afforded by 7.5 to 15 Sherman vitamin B_1 units daily. About two-thirds protection, as judged by gains in body weight, was possible with a daily allowance of 15 units of vitamin B_1, when the daily

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* Research paper No. 381, Journal Series, University of Arkansas, Fayetteville.

[1] Sure, B., and Smith, M. E., *J. Nutr.*, 1934, **7**, 547.

[2] Sure, B., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 779.

thyroxin dose was 0.1 mg. A daily dose of 30 vitamin B_1 units prevented loss of weight but permitted only little growth when the daily thyroxin allowance was 0.2 mg.

Crystalline Vitamin B_1. While 10γ (0.01 mg.) of crystalline vitamin B_1 seems to antagonize satisfactorily a 0.05 mg. thyroxin dose, complete protection against 0.1 mg. and 0.2 mg. thyroxin has not as yet been reached by as much as a 300γ (0.3 mg.) daily dose of crystalline vitamin B_1.†

Since 1 Sherman unit is equivalent to 2.5γ of crystalline vitamin B_1,[3] it has been possible for us to make observations on the biological value of the Lilly vitamin B_1 concentrate and the Merck's crystalline vitamin B_1, as indicated by unit doses. Since we are unable to secure the same increments of growth on higher doses of crystalline vitamin B_1 on our vitamin B_1 deficient diet[1] as Waterman and Ammerman,[3] and since there seems to be a supplementary relationship between the Lilly vitamin B_1 concentrate and the Merck's crystalline vitamin B_1, the evidence points to the existence of an essential component of the vitamin B complex for the mammalian organism other than vitamin B_1 and vitamin B_2 (the latter being furnished in abundance in our diet by 15% autoclaved beef),—possibly B_4, as originally suggested by Reader.[4]

8257 P

Enzymic Efficiency in Avitaminosis.*

BARNETT SURE, M. C. KIK, AND K. S. BUCHANAN.

From the Department of Agricultural Chemistry, University of Arkansas.

In previous studies[1] we found that in vitamin B deficiency there is a marked reduction in the efficiency of digestion of pancreatic lipase but no demonstrable disturbance in the activity of either trypsin or erepsin. We have now completed considerable work with other enzymes in vitamin A as well as vitamin B deficiency. A pre-

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† Since this material went to press, we have found that a sufficiency of yeast in the diet or administered separately from the ration will antagonize the toxicity of as high a daily dose of thyroxin as 0.2 mg. to the extent of 75 to 100 per cent.

[3] Waterman, R. E., and Ammerman, M., *J. Nutr.*, 1935, **10**, 38.

[4] Reader, V., *Biochem. J.*, 1929, **23**, 689; *Ibid.*, 1930, **24**, 77, 1827.

* Research paper No. 382, Journal Series, University of Arkansas, Fayetteville.

[1] Sure, B., Kik, M. C., and Buchanan, K. S., *J. Biol. Chem.*, 1935, **108**, 19.

liminary report at this time might, therefore, be of interest to other workers in this field.

The biological technique employed was the same as previously described.[1] The methods of titrating the enzymes were outlined recently.[2]

The results can be best presented by submitting summarized data in tabular form. Various stages of avitaminosis were studied. The following tables, however, represent general summaries of average results for all the different stages of vitamin deficiencies.

TABLE I.

% Changes† in Concentration of Various Blood and Tissue Enzymes in Vitamin B_1 Deficiency Compared with Litter Mate Controls of the Same Sex Restricted to the Same Diet and Same Plane of Nutrition.

Enzyme		No. of Groups	Enzyme		No. of Groups
Pancreatic esterase	—40.5	18	Bloom serum amylase	—9.7	17
” lipase	—26.8	18	Hepatic esterase	—1.1	17
Hepatic lipase	—18.3	18	Pancreatic amylase	—0.7	18
Blood serum esterase	—13.3	18	Blood serum phosphatase	+21.0	16

† + = increase, — = decrease.

TABLE II.

% Changes† in Concentration of Various Blood and Tissue Enzymes in a Deficiency of Vitamin B Complex Compared with Litter Mate Controls of Same Sex Restricted to Same Diet and Same Plane of Nutrition. (9 Groups Studied.)

Enzyme		Enzyme	
Pancreatic esterase	—42.2	Pancreatic amylase	—2.5
Pancreatic lipase	—19.7	Blood serum phosphatase	+58.0
Blood serum esterase	—14.2	Hepatic lipase	+17.5
Blood serum amylase	—6.5	Hepatic esterase	+15.2
Kidney phosphatase	—4.9		

† + = increase, — = decrease.

TABLE III.

% Changes in Concentration of Various Blood and Tissue Enzymes in Vitamin A Deficiency Compared with Litter Mate Controls of the Same Sex Restricted to Same Diet and Same Plane of Nutrition.

Enzyme		No. of Groups	Enzyme		No. of Groups
Blood serum esterase	—40.2	33	Pancreatic lipase	—4.4	36
Hepatic esterase	—15.6	36	Pancreatic esterase	—2.2	35
Blood serum amylase	—13.6	32	Pancreatic amylase	—0.1	37
Erepsin	—10.1	32	Hepatic lipase	+35.0	35
Blood serum phosphatase	—6.2	33	Trypsin	+0.9	37

An examination of the tabular data discloses that the most marked changes in vitamin B_1 deficiency are decreases in pancreatic lipase and pancreatic esterase activity. In a deficiency of the vitamin B complex, in addition to considerable decrease in efficiency of pancreatic lipase and esterase, a pronounced increase in concentration of blood serum phosphatase was observed. In vitamin A deficiency the most outstanding feature is the large decrease in concentration of blood serum esterase and the increase in hepatic lipase.

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[2] Sure, B., Kik, M. C., Buchanan, K. S., Thatcher, H. S., and DeGroat, A., *Biochem. J.*, 1935, **29**, 1508.

In so far as the relation of vitamins to digestion is concerned, the only influence found to date is the marked decrease in efficiency of digestion of fats in vitamin B_1 deficiency. We found no disturbance in digestion of proteins or starches in either vitamin A or vitamin B deficiency.

8258 P

Experimental Formation of Accessory Organs in Mid Body Lateral-Line of Amphibians.*

L. S. STONE.

From the Department of Anatomy, Yale University School of Medicine.

The ability of primary lateral-line organs in amphibians to form accessory ones by budding,[1, 2] the time at which these bud organs are developed, and the pattern of organ groups gave a method for measuring the effects of the present experiments in amphibians.

When the placodes of the mid-body lateral-line primordia of young and older tail-bud stages of *Amblystoma punctatum* (Harrison stages 23-24 and 28-29) are exchanged, the age difference between graft and host organ-forming tissue can be clearly followed through the young larval stages by observing the time at which accessory organs are laid down by the budding of primary ones.

In *A. punctatum* the dorsal and ventral poles of primary organs give rise to accessory organs forming lineal groups of 2 or 3.[1] In *A. tigrinum* by a similar process groups of from 4 to even 8 are formed, usually in irregular clusters, as in *Rana palustris* and *Hyla crucifer*. There is multiple budding of both primary and accessory organs. When the mid-body lateral-line primordia are exchanged in the tail-bud stages of *punctatum* and *tigrinum* the formation and grouping of organs from the graft follow only that of the donor species.

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* These studies were supported by financial aid from the Fluid Research Funds of the Yale University School of Medicine.

[1] Stone, L. S., *J. Comp. Neur.*, 1933, **57**, 507.

[2] Stone, L. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1082.

When the lateral-line primordium of *A. punctatum* is placed on the body of *A. tigrinum* in the future pathway of the primordium of the host, the one of the graft will migrate and form organs only when the primordium of the host touches and fuses with similar material of the graft. The phenomenon is similar to that previously reported.[3] At the appropriate times accessory organs are developed, which in size and group formation are identical with those of the donor species. The organs are innervated by the host lateral-line nerve and never were observed to degenerate up to late larval life.

When a portion of the tail in larvae is amputated the terminal organ on the stump gives rise to a chain of organ-forming cells extending posteriorly into the regenerating tail. The number of new organs slightly exceeds the number removed. In older larvae either a primary or one of its accessory organs gives rise to the regenerating chain, but no more than one organ in such a group has been seen to participate at one time. This holds true for both the anurans and urodeles studied.

When skin on the side of the tail in young *A. punctatum* larvae is excised, including from 1 to 3 organs, the primary organ just anterior to the wound area often gives off at its posterior pole a chain of cells which form 1 to 2 organs in the wound-healed region. This is the same general effect as that following amputation of the end of the tail. In no case did the anterior pole of the organ just posterior to the wound give rise to a bud organ.

To demonstrate whether or not the anterior pole of a primary organ contains supporting cells which are potential for new organ-formation, another experiment was devised. The technique will be described in a later publication. Two to 3 mm. of the end of the tail were excised in each of a pair of young *punctatum* larvae. The wound areas were healed to each other in the manner of parabiosis. In about 2 days the animals were cut apart so that about 1.5 mm. of the stump of the tail of one larva was left attached to that of the other. In this manner, then, a graft was made of a segment of the tail reversed 180° anteroposteriorly. The former anterior pole of the terminal organ of the graft was then directed posteriorly toward the cut edge from which a regenerating tail could develop.

From this terminal organ in the graft a chain of many bud-organs was produced which extended into the regenerating tip of the tail graft. The original anterior pole of the primary organ is, as all other poles, potential for organ formation, the test for which the experiment was devised.

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[3] Stone, L. S., *Anat. Rec.*, 1928, **38**, 30.

The former posterior pole of the terminal organ of the graft, now anteriorly directed, may at the same time give rise to a chain of organs extending anteriorly in the rapidly growing graft. From the 2 poles in such a terminal organ the line of new organs may equal in number that of the primary organs found in the entire tail on one side of a normal young larva of *A. punctatum.*

The terminal organ of the host may give rise to a chain of organs extending posteriorly into the region of the graft, a reaction similar to simple amputation of the tail tip. The organ of the graft nearest the host tissue may also give off bud-organs anteriorly (from former posterior pole) to meet such a chain from the host. This seems to depend upon the direction and extent of the early regeneration in host and graft tissue.

8259 C

Caseation and Softening in Tuberculosis of Vaccinated and Unvaccinated Guinea Pigs.

JOSEPH D. ARONSON.

From Phipps Institute, University of Pennsylvania.

There is much evidence that vaccination with the BCG strain of the tubercle bacillus produces a certain degree of resistance to reinfection with virulent tubercle bacilli. The question has been raised, however, as to whether the allergy resulting from such vaccination might be a factor in producing a more extensive and more destructive type of tuberculosis. In an experiment to ascertain if any relationship exists between allergy and the character of the tuberculous lesions, one series of guinea pigs was injected subcutaneously in the left inguinal region with 5 mg. of the BCG culture, a second series was injected in a similar manner with 30 mg. of the virulent Ravenel strain of bovine tubercle bacillus killed by heating for 1 hour at 70°C., and a third group of unvaccinated guinea pigs were used as controls.

Six weeks after vaccination the sensitivity of these animals to old tuberculin was determined by injecting into the skin of each animal, at widely separated points, 1, 5 and 10 mg. of old tuberculin, and reading the results 48 hours later. It was found that among the 14 guinea pigs of the first series, injected with BCG, 9 reacted to 1.0 mg. of tuberculin and 5 to 5.0 mg. Of the 15 guinea pigs of the second series, injected with the heat-killed tubercle bacilli, 3 reacted to 1.0 mg. of tuberculin, 6 to 5.0 mg., 5 to 10.0 mg., and one failed to react. The 11 untreated control guinea pigs did not react to the injection of tuberculin. The vaccinated as well as the unvaccinated control animals were then injected intratracheally while under ether anesthesia with approximately 100 tubercle bacilli of the virulent Ravenel bovine strain. Six weeks later all of the animals were killed and necropsied.

Results in Guinea Pigs Vaccinated with BCG Cultures. The cervical lymph nodes were tuberculous in but one animal of this group. The hilum lymph nodes, which were enlarged in all the pigs, measured on an average 12x7x4 mm. They were firm and fibrous, except in an occasional animal in which they were fibrocaseous. Microscopically these nodes showed marked increase of the stroma and the capsule was thickened and fibrous. Widely separated small areas of caseation were noted in the sections of most animals, but in an occasional animal caseation was extensive and confluent.

The lungs contained a small number of discrete tubercles ranging from less than 1 to 2 mm. in diameter. These tubercles were most numerous in the lower lobe, and only occasionally was caseation noted macroscopically. Microscopically these tubercles consisted of either a diffuse intra-alveolar infiltration of large mononuclear cells or of aggregations of these cells about the smaller bronchi. In 7 of the 14 guinea pigs of this series small areas of caseation surrounded by epithelioid cells were observed.

The spleens, slightly larger than normal, measured on an average 27x14x2 mm. In 3 of the animals a small number of discrete minute tubercles were seen macroscopically. Microscopic examination of the spleens showed that in many instances the Malphigian corpuscles and germinal centers were hypertrophied. The splenic pulp contained an increased number of large mononuclear cells. In the spleen of 3 animals a number of epithelioid tubercles were observed, with small caseous centers. In the liver of one animal a small tubercle was noted, while in another animal the periportal lymph node was fibrocaseous.

Tubercle bacilli were not demonstrable in any of the lesions, nor was softening observed in any of the tuberculous tissue studied.

Results in Guinea Pigs Vaccinated with Heat-killed Tubercle Bacilli. The cervical lymph nodes were found to be enlarged and caseous in 5 of 15 guinea pigs. The hilum lymph nodes, which were enlarged and caseous, measured on an average 23x12x7 mm. Microscopically these nodes showed extensive and confluent caseation associated in 13 instances with softening. This softening was characterized by definite dissolution and separation of the necrotic material from the adjacent tissue, resulting in an abscess-like formation. In 8 instances tubercle bacilli were found. In the lungs there were observed numerous tubercles 3 to 5 mm. in diameter, many of which were caseous. Microscopically the tubercles, usually peribronchial, consisted of extensive areas of caseation surrounded by numerous epithelioid cells and a small layer of lymphocytes at the periphery. The alveoli were filled with epithelioid cells, the lymphatics were dilated, and conspicuous aggregations of lymphocytes were noted about the larger blood vessels. In a small number of animals extensive edema was present. Softening of the caseous areas was observed in the lungs of 11 animals, and tubercle bacilli were found in 8 instances.

The spleens were markedly enlarged, averaging 38x20x4 mm. in size. They were dark red or purplish and their surfaces were mottled by grayish areas of caseation. Microscopically a marked infiltration of epithelioid cells and numerous giant cells were noted. Caseation was extensive and, in some instances, confluent. Softening was observed in 3 instances, while tubercle bacilli were demonstrated in the spleens of 6 animals. In 13 of the 15 pigs, the liver was found to have an occasional tubercle, and in the remaining 2 animals tuberculosis was extensive. The periportal lymph nodes were enlarged and caseous in every animal.

Results in Unvaccinated Guinea Pigs. The cervical lymph nodes were enlarged and caseous in 6 of the 11 unvaccinated control animals. The enlarged caseous hilum lymph nodes averaged 21x12x7 mm. Microscopically the pathological findings resembled those noted in the hilum lymph nodes of the animals injected with the heat-killed tubercle bacilli, but the caseation was more extensive. Softening was observed in 8 instances and tubercle bacilli were demonstrable in 6 instances. In the lungs a number of tubercles ranging in size from 3 to 5 mm., and in some instances larger wedge-shaped confluent tubercles measuring 10 to 15 mm., were noted. Microscopic examination revealed areas of extensive caseation, some of which were confluent. Epithelioid cells were noted in large numbers about the caseous centers and in the alveoli. The tubercles were located about the smaller bronchi and beneath the pleura. Softening and tubercle bacilli were observed in 7 instances. In some animals there was noted a diffuse edema. In general the tuberculous process was more extensive in this series of guinea pigs than among those vaccinated with either the BCG culture or with the heat-killed cultures of bovine tubercle bacilli.

The spleens, which were all enlarged, measured on the average 30x23x3 mm. In most instances they were studded with macroscopic tubercles of varying size. Microscopically numerous tubercles consisting of extensive areas of caseation were found; many of them confluent. Numerous epithelioid cells were observed about the caseous areas, as well as in other parts of the spleen. Softening of the tubercles was observed in 2 instances, and tubercle bacilli were demonstrable in the spleen of 6 animals.

Summary. Guinea pigs vaccinated with the attenuated BCG strain of tubercle bacillus, and thereby made allergic, were reinfected intratracheally with a small number of virulent tubercle bacilli. Examination of the animals 6 weeks after reinfection showed a small number of fibrous or fibrocaseous tubercles in the organs and lymph nodes in most instances, but failed to show any evidence of softening, nor could tubercle bacilli be demonstrated in the sections. Guinea pigs vaccinated with heat-killed tubercle bacilli, although less allergic, as well as control, unvaccinated guinea pigs that were not allergic, when reinfected with virulent tubercle bacilli showed 6 weeks after reinfection extensive tuberculosis of the organs and lymph nodes with well marked softening; and in most instances numerous tubercle bacilli were demonstrable in the sections. Thus, no relationship could be established between the presence of allergy at the time of reinfection and the subsequent softening of the tuberculous process.

8260 P

Nitrogen Content of the Bile of Normal and Diseased Gall Bladders.*

H. G. ARONSOHN AND E. ANDREWS.

From the Department of Surgery, The University of Chicago.

A series of recent papers have indicated the possibility that simple bacterial infection is not sufficient to explain the onset of at least part of the clinical cases of cholecystitis. Not only has the diseased gall bladder in about half the cases defied the attempts of bacteriol-

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

ogists to grow organisms from it, but, as Feinblatt[1] and others have pointed out, the histological picture is not consistent with that to be expected in a purely infectious process. The evidence in this matter has recently been summed up by one of us.[2, 3] With these points in view the following series of studies of the possible or probable chemical constituents of the bile has been undertaken in order to elucidate the possibilities of a purely chemical cholecystitis.

While each of the normal constituents of bile has at some time or other been accused as the causative agent of cholecystitis in man, very little work has been done on its nitrogen content. The striking conclusions found by Gundermann[4] have received but little attention. He found that the total nitrogen content of the human gall bladder was much higher in cholecystitis while in stone-containing gall bladders, it was variable. Boekelman,[5] by aspirating human bile from the duodenum, arrived at similar conclusions.

TABLE I.

	No. of Cases	Total N, Mg. %		Prot. N, Mg. %		NPN, Mg. %	
		Range	Aver.	Range	Aver.	Range	Aver.
I. Stone containing	5	315-577	496.2	93.8-170.5	118.2	162-442.5	326.5
II. Chronic changes on the gall-bladder wall (adhesions, hypertrophy, obliteration, etc.)	21	55-924	437.3	40-462	191.5	37.4-444	229.2
III. Normal and distended gall-bladders	15	43.1-798	452.1	43.1-375	158.2	12.3-600	207.1

41 human postmortem biles.

Table I shows the range and average figures of 41 postmortem biles and it is obvious that there is no significant relationship in the different groups. Lack of space prevents us from giving the individual figures but suffice it to say that their fluctuations are extreme and apparently pointless.

Table II gives the data on bile from 13 human gall bladder operations from which more accurate clinical and microscopic data could be secured. No definite conclusions can be drawn from these results. The figures in the different groups vary widely and no characteristic level for any group may be postulated.

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[1] Feinblatt, H. M., *New Eng. J. Med.*, 1928, **109**, 1073.

[2] Andrews, E., and Dell, L., *Arch. Int. Med.* In press.

[3] Andrews, E., *Trans. West. Surg. Assn.*, 1934.

[4] Gundermann, *Mitt. a. d. Grenzgeb. d. Chir.*, 1926, **39**, 353.

[5] Boekelman, *Klin. Wochen.*, 1928, **7**, 65.

TABLE II

	No.	Mg. % Tot. N	Mg. % Prot. N	Mg. % NPN
Gall bladder bile				
Stone-containing bile				
Acute change (round cell infilt., edema, etc.)	1		521.4	
	2		28.0	
	3	24	10.7	
	4	207	111.0	88.0
Chronic change (hypertrophic processes, fibrous thickening, etc.)	5		37.5	
	6	75	34.1	41.6
	7		pract. none	
	8	285	136.5	125.2
	9	251	115.0	125.0
About normal (very little cell reaction) distended gall bladder	10	222	30.0	
	11	157	55.0	
	12	414	222.0	157.0
Liver bile	13	44	12.0	28.0

13 human operation biles.

For the sake of comparison the average figure for dog biles taken from the gall bladder was found to be 340 mg. of total nitrogen, while in experiments where the common duct was ligated, it was found that in some cases this total nitrogen rose and in others it fell, there being no consistency in its fluctuation even if several weeks elapsed.

8261 P

Bile Salt Cholecystitis.[*]

H. G. ARONSOHN AND E. ANDREWS.

From the Department of Surgery, The University of Chicago.

Pancreatic juice, in the case of obstruction at the ampulla of Vater may become activated by some unknown factor and develop a marked proteolytic activity in the pancreatic tissues. In the same manner the digestive power of gastric juice may become so enhanced that a gastric ulcer will be produced. Whatever other factors are active, it is clear that pure gastric juice *per se* may have the power to erode the gastric mucosa. It has been suggested recently

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[*] This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

by one of us[1] that similar respect may be due the bile and that it may under certain circumstances acquire a destructive power in the same manner as the gastric juice. The most obvious constituent of the bile which has a toxic and destructive power on body tissues is of course the bile salts, which may exist in solution in the bile in concentrations higher than any other material in the body fluids. As the bile is concentrated in the gall bladder the bile salt content frequently rises to 7 or 8%. While our own studies have indicated that these figures are seldom exceeded, the analyses given in two textbooks of physiological chemistry, Hawk's[2] and Hammarsten's,[3] give the normal concentration of bile salts at about 10%.

Protocols. Two forms of bile salts were used: (1) so-called Purified Bile Salts (Armour and Company); (2) the same material freed by alcohol extraction from the very large percentage of non-alcohol-soluble material which it contains. The injections were all made by the non-traumatic technique discussed above. Twenty-two dogs were operated upon, six of these serving as controls. Sixteen dogs received 20-35 cc. of 10-15 or 20% bile salts. The controls consisted of the introduction of the catheter alone (4 dogs) and the introduction of a catheter plus a washing of the gall bladder with normal salt solution (2 dogs). All the controls showed normal gall bladders with the exception of one which revealed a little bleeding on the serosa. The purified bile salt injections, in every concentration used, gave inflammatory reactions of the gall bladder. The commercial bile salt preparation gave positive results regularly in a concentration of 15-20%, whereas in a concentration of 10% the results were two positive and two negative. Thus the purified form seems the more active.

The result of these injections was the production of an acute cholecystitis more closely resembling that found in the human being than any the authors have ever encountered experimentally. The gall bladders were enormously enlarged and very hard, while the cystic ducts were obviously sclerosed and shut. The color of the gall bladder was greyish-white, streaked with hemorrhages and bile-stained spots, as found in the acute form of human cholecystitis. Several of the dogs died of bile peritonitis without any demonstrable macroscopic opening in the gall bladder. The adjacent viscera in many cases were fastened to the gall bladder by fresh, fibrinous adhesions. The gall bladder walls were as much as 7 or 8 mm. thick, and stiff and unbending. On microscopic examination the mucosa appeared normal or as nearly so as it usually does in human cholecystitis. The muscularis was moderately edematous and contained a considerable number of large and small mononuclear cells with a marked scarcity of polymorphonuclear leucocytes. The reaction appeared to take place mostly in the serosa which was 8 to 10 times its normal thickness, being tremendously edematous, showing marked hyperemia and a number of punctate hemorrhages, together with the characteristic dilatation of lymphatics and moderate infiltration of round cells. The picture was similar but very much more marked than that produced by protein injection and resembled with a surprising accuracy that of acute human cholecystitis, differing markedly from the effects of bacterial injection.

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[1] Andrews, E., *Surg. Gyn. Obst.*, 1935, **60**, 239.

[2] Hawk, Practical Physiological Chemistry.

[3] Hammarsten, O., Textbook of Physiological Chemistry.

Considering the relatively slight increase in bile salt concentration beyond the 6-10% supposed to be normal for the human, these results may be looked upon as decidedly significant and further experiments are being undertaken to investigate the effect on this phenomenon of changes in the acid base equilibrium, variations of the osmotic pressure of its contents and other factors which might influence it.

8262 P

Non-Bacterial Cholecystitis. The Mechanism of Acidification of Bile in the Gall Bladder.*

H. G. ARONSOHN and E. ANDREWS.

From the Department of Surgery, The University of Chicago.

Okada[1] called attention to the marked acidification of the bile during its concentration in the gall bladder. However, the mechanism by which this acidification takes place has never been completely elucidated. Ravdin[2] studied the question and found that dog's liver bile had a pH of 7.1 or even higher and attributed the acidification in the gall bladder to some unknown anion. This present study has consisted of parallel analyses of the gall bladder and liver bile

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* This work was done in part under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

[1] Okada, S., *J. Physiol.*, 1915, **1**, 114.

[2] Ravdin, G. G., Johnstone, C. G., Riegel, C., and Wright, L. L., *Am. J. Physiol.*, 1932, **100**, 317.

TABLE I.

	pH	CO_2	Mg. % Cl	Mg. % B.A.	Mg. % P	M Eq / L Base	Mg. % Ca	Mg. % Mg	Mg. % Tot. N	Mg. % NPN	Mg. % Prot. N	G. % Solids
Liver bile	6.71	95.5	243.6	204.5	27.5	187.5	8.8	pract. none	178.4	12.4	166.1	6.6
Gall bladder bile	5.96	20.5	79.8	719.5	200.9	211.2	24.3	pract. none	382.7	43.5	339.1	21.1
Ratio of concentration	—	0.21	0.32	3.6	7.3	1.1	2.8	—	2.2	3.5	2.0	3.2

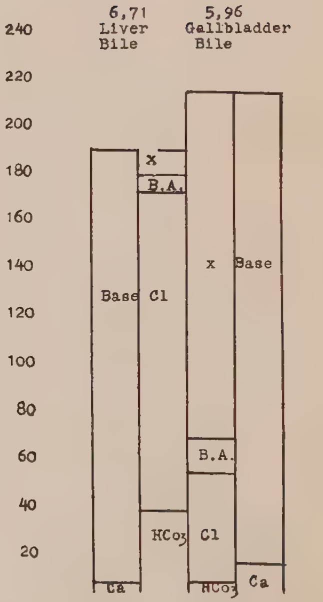

CHART 1.

in which all of the principal factors affecting the acid-base equilibrium were estimated. (Chart I.)

Special stress is laid on the method of obtaining the bile because in many of the previously reported cases the effects of anesthesia and other extraneous phenomena could not be ruled out. In our dogs the liver bile was collected after first performing a laminectomy in the mid-dorsal region under local anesthesia and a trans-section of the spinal cord. Following this, when the dog had recovered from the shock, a laparotomy was performed, the cystic duct clamped, and the common duct cannulated, the cannula being connected with a tube leading to a glass container in which the bile was collected under oil. This elaborate technique was necessary as repeated attempts to secure bile under a number of different anesthetics showed us that the quality of the bile was markedly affected by the drug. This technique gave us a constant flow of clear bile without any admixture of air or blood, Special care was taken to determine the carbon dioxide and pH of the bile immediately. Standard methods of blood and urine analyses were used throughout except with the phosphorus, for which the specimens were ashed in a muffle furnace.

The averaged analytical results from 7 liver and 12 gall bladder biles are reported in the chart. The very high carbon dioxide content of liver bile (confirmed by a series of 30 separate analyses) undoubtedly explains why the pH level is distinctly lower than that reported by previous investigators,[2] who omitted the same precaution to avoid loss of gas. In the gall bladder bile the low CO_2 value is a reflection of the decreased pH. The concentration of total base is essentially unchanged, while the considerable loss of chloride is more than compensated by an even more marked rise in phosphorus content. This last, in conjunction with the increase in concentration of protein (which must possess notable base-binding power), therefore accounts for the acidification of the gall bladder bile. The rise in bile-acid concentration also contributes slightly to this effect.

8263 C

Experimental Necrotizing Arteriolitis Induced by a Protein Cleavage Product.

RALPH R. MELLON, MARGARET R. BAKER, AND ALICE P. McILROY.

From the Western Pennsylvania Hospital, Institute of Pathology, Pittsburgh.

With K. F. Semsroth, formerly of this Institute, one of us (Mellon) initiated experiments that have been substantially amplified and extended through the assistance of the above collaborators. The few original experiments were the outgrowth of unpublished studies by one of us (Mellon), attempting to correlate the antiseptic action of iodine with its wound healing power—the inference appearing justified that it might be partially accounted for by its neutralization of certain toxic products of protein decomposition, originating perhaps from the tissues or from microorganisms. In support of this point of view Eppinger,[1] found that iodine neutralized the toxicity of putrifying meat pulp. He inferred from his experiments that the toxicity was due to certain unsaturated protein cleavage products, of which allylamine is a type. From our standpoint, the pertinent pathological effect of allylamine as shown by him is a marked increase of endothelial permeability.

It was, therefore, natural to see whether allylamine applied to wounds would delay their healing, and to observe whether the effect on the endothelium was reflected in detectable morphologic alterations in the vessel walls. Both effects were observed, the latter being of a noteworthy character, consisting in their close similarity to the experimental arteriolitis of Klinge[2] as shown in his hyperergic vascular lesions, and to the necrotizing arteriolitis and malignant nephrosclerosis of Fahr.[3]

Twenty-one rabbits were employed. The allylamine was applied to small areas denuded of skin, as well as given intradermically. Control administration of NH_4OH was employed. Each type of solution, the allylamine and the NH_4OH, was used in both buffered and unbuffered form. All buffered solutions had a pH of 7. The doses of allylamine ranged from 0.2 cc. to 0.5 cc. (intradermically) of solutions varying in concentration from 1% up to one to one million (10^{-6}). Vascular changes were discernible in concentrations as low as 10^{-5}, although they were more characteristic at 10^{-3} and 10^{-4}.

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[1] Eppinger, H., *Klin. Woch.*, 1935, **13**, 1105.

[2] Klinge, *Ergebn. d. Allg. Pathol.*, 1933, **27**.

[3] Fahr, Th., Henke-Lubarsch, Handbuch. d. spec. pathol. Anat., Vol. VI.

This was the case for even the buffered solutions, none of which caused macroscopic lesions in dilutions of more than 1:100. Although the unbuffered NH_4OH controls caused local sloughing, especially in higher concentrations, in only an insignificant percentage of lesions was a vascular effect detected—unless employed on the same animal as the test substance. For example, when histamine was employed as an additional control, the unbuffered NH_4OH yielded at times an appreciable percentage of lesions.

The histamine dosage ranged from 10^{-2} to 10^{-5} cc., 0.2 cc. of each dilution being employed. Both buffered (pH 7) and unbuffered solutions were used. Vascular effects were absent in *all dilutions of the buffered material;* but with the unbuffered, the characteristic microscopic effects were observed in solutions no more dilute, however, than in 1×10^{-3}. Macroscopic effects were also noticeable in the unbuffered, but not in the buffered solutions. In this connection it is of interest that histamine has in its molecule the allylamine linkage. Moreover, control observations with allylformate and allylalcohol indicated the paramount importance of the amine group.

In its earlier stages an outstanding feature of this arteriolar disease is first: hemorrhage into the wall; and second, a loss of its structure accompanied by the presence of a homogeneous, highly eosinophilic material. (Fig. 1.) In its later stages a definite proliferative endarteritis is often seen, even to obliteration of the ves-

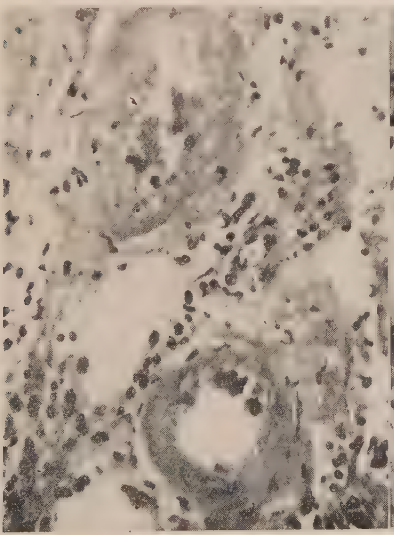

FIG. 1

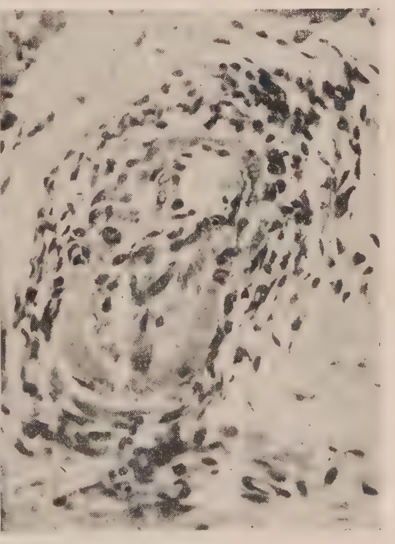

FIG. 2.

sel's lumen. Perivascular changes consist of mononuclear infiltration of tissue polyblasts (Fig. 2), as well as the formation of a nodular periarteritis (Figs. 3 and 4). Fig. 3 represents a nodule whose continuity with the adventitia of the occluded vessels seen in Fig. 4 was traced by serial section. Thus the process recalls a periarteritis nodosa. Note also in Fig. 2 that the lower segment of the vessel shows the intramural change, while in the upper portion the perivascular infiltration of mononuclear cells dominates.

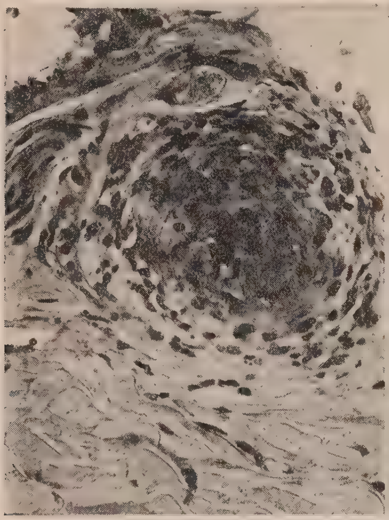

Fig. 3.

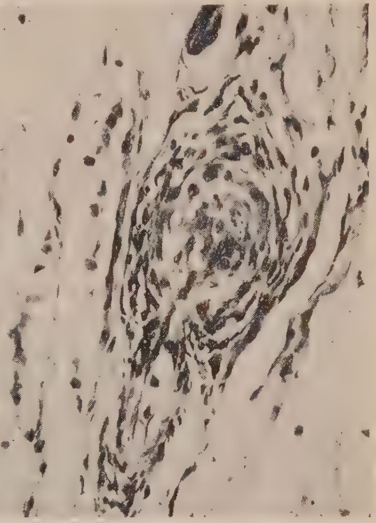

Fig. 4.

Endarteritic, as well as periarteritic, processes are *suggestively sequelae* of the appearance within the vessel wall of the homogeneous eosinophilic material described above. The coincidence of the latter with endothelial necrosis and intramural hemorrhage in early, rather than late, stages, the absence of nuclear fragmentation within the homogeneous matter in the early stages, its extension beyond the arteriolar walls, suggest that this intramural homogeneous material is composed of coagulated blood plasma. Weigert's fibrin stain, silver stains, and Mallory's anilin blue support this impression that the substance is fibrin or fibrin-like. Its presence in the arteriolar wall obscures and probably partially destroys the normal mural structures.

Summary. From these results it appears justifiable to draw tentatively the following inference: the intracutaneous injection of allylamine in rabbits leads to a necrotizing arteriolitis strikingly similar to that conventionally present in certain human infections (the streptococcoses especially) which have prominently in their background the phenomena of hypersensitiveness. In the pathogenesis of the latter a primary endothelial damage making for increased permeability appears to be of significance.

Histamine, the substance par excellence from which one might expect such effects, produces it only in relatively high concentrations; and only in the unbuffered form. As far as we are aware such vascular changes have not been described for protein cleavage products of relatively simple constitution; particularly in rather high dilution and of a neutral pH. In a physiological sense the allylamine is to be looked on as histamine-like, at least in its vascular effects. But it is decidedly more active in this respect, even under pH conditions prevailing in the host where histamine has exhibited no effect as far as the studies have progressed.

8264 C

A Sex Difference in Gonad-Stimulating Potency of Young Gonadectomized Rats.

KATHRYN F. STEIN. (Introduced by A. E. Adams.)

From the Zoology Department, Mount Holyoke College.

Clark[1] recently reported that in rats gonadectomized at 1 or 2 days of age and assayed 16 to 18 days later the gonad-stimulating potency of the pituitaries was approximately the same in both sexes. Since in normal control animals of that age (17 to 20 days) the sex-stimulating potency of the female pituitary exceeds that of the male, she concluded that castration at 1 or 2 days produced a relatively greater increase in gonadotropic potency than spaying. The following series of preliminary experiments indicates that if gonadectomy is delayed for a week the pituitary of the castrated male does not gain as much in gonadotropic potency as that of the spayed female in the same period of time.

Littermate male and female rats 7 to 9 days of age were gonadectomized and after 17 to 19 days their pituitaries were injected into immature female rats 23 to 26 days of age. Normal males and females, littermates of the gonadectomized series, served as control donors. A host series consisted of 4 littermate females. Three donor glands were weighed, minced, and injected by means of a fine glass pipette, according to the method of Smith and Engle,[2] into the leg muscle of each host. The hosts, and usually a control littermate, were killed 4 days after injection and the ovaries weighed and fixed. Various factors intervened to prevent obtaining complete results for all series. Donors often were not born in adequate numbers of both sexes, maternal cannibalism caused blanks in several series, and in a few cases fatalities occurred among prospective or actual hosts. Out of 14 series, full results were obtained in only 4, and the present report is limited to these with the exception of case 14 for which the hosts, although not littermates, were of the same age and very uniform in body weight. Table I gives the data for the 5 series. The inclusion of all fragmentary series with the present data would not change the general results.

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[1] Clark, H. M., *Anat. Rec.*, 1935, **61**, 193.

TABLE I.

Wt. of injected pituitaries in mg.					Actual wt. of host ovaries in mg.					$\frac{\text{Wt. of host ovaries in mg.}}{\text{Body wt. in gm.}} \times 100$				
No.	♂	♀	♂*	♀†	Control	♂	♀	♂*	♀†	Control	♂	♀	♂*	♀†
4	6.6	6.8	9.8	8.8	14.3	9.5	10.4	28.8	44.2	27.5	20.2	21.2	68.6	86.7
11	5.6	6.8	7.5	9.4	10.0	15.4	14.3	9.4	41.2	40.0	44.0	37.6	30.3	125.0
14	5.6	7.0	8.4	9.2	12.2	14.0	14.0	30.2	77.2	22.2	26.6	25.7	50.3	138.0
17	5.0	6.0	9.2	9.0	14.0	16.2	23.4	17.2	30.6	25.9	29.3	43.3	31.3	61.2
19	5.3	6.0	8.4	8.0		14.8	15.6	14.0	48.8		29.6	29.7	34.1	90.4
Av.	5.6	6.5	8.7	8.9	12.6	13.98	15.5	19.9	48.5	28.9	29.9	31.5	42.9	100.7

*Castrated. †Spayed.

Both in percent of body weight and in actual weight the ovaries of animals injected with pituitaries of spayed females were heavier than those injected with glands of castrated males. This is true for each individual series as well as for the average of all series. The averages also show greater potency in glands of normal females than in those of normal males. The ovaries of animals injected with normal male glands are on the average 90.2% as heavy as those of animals receiving normal female glands. As a figure for donors of 25 to 27 days this is comparable to one of 88.3% similarly obtained from Clark's[3] data for 20- to 25-day donors.

Clark[1] suggests that castration of the male at 1 to 2 days of age withdraws a much larger amount of sex hormone from the animal than spaying of a female of the same age. In the male, gonadectomy then removes a more powerful influence and allows the pituitary to show a greater increase in potency relative to that exhibited by the spayed female. The apparent reversal of this condition if gonadectomy is delayed for one week may perhaps indicate that during the first week of postnatal life the testis exerts upon the pituitary a definitive influence the effects of which are not eradicated by a 17- to 19-day absence of the gonad. In a recent abstract Pfeiffer[4] reports a sex differentiation in the hypophysis which is not present at birth but which once established cannot readily be altered. Perhaps a critical stage in the period of differentiation is the first week after birth. Histological work and further experiments are planned for a study of this problem.

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[2] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1927, **40**, 159.

[3] Clark, H. M., *Anat. Rec.*, 1935, **61**, 175.

8265 C

Amount of Progestin in Corpora Lutea of Cows.

GIMBO G. KIMURA. (Introduced by George W. Corner.)

From the Department of Anatomy, The University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Practically all work dealing with the corpus luteum hormone, progestin, has been done with material derived from the pig, and most of the determinations of yield which give us information as to the amount of hormone in the raw material have been made on that species. A few investigators however have used corpora lutea of the cow and this source appears to be more convenient in some parts of the world. It may be of interest, therefore, to report the result of a brief study of the amounts of progestin found in cows' corpora lutea.

Three different collections of fresh cows' corpora lutea were made at different times: 2 from the local slaughter houses and one from the Wilson Laboratories of Chicago. In selecting the corpora lutea from the freshly slaughtered cattle, only the active glands of pregnant and non-pregnant cows were used, including the first stage in which the color is pale and stained with blood, and later stages marked by a cream color changing to bright yellow, and finally to reddish orange. Glands showing signs of retrogression, "corpora rubra," identified as being bright red to brown in color, and toughened by the presence of much connective tissue, were all discarded.

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[4] Pfeiffer, C. A., Advance Abstract Sheet, Wist. Inst. Bibl. Ser., July 15, 1935, 6.

The material was minced and stored in 2 volumes of 95% alcohol, Allen having observed[1] that it may be so stored for at least 10 months with no loss of activity.

Alcoholic extracts were prepared following the procedures of Corner and Allen,[2] and standardized by their method, one rabbit unit being the minimum amount necessary to produce a +++ or ++++ progestational proliferation of the uterine mucosa of a freshly spayed adult female rabbit. The test is described in detail by Corner and Allen[2] and illustrated by Allen.[1] The length of time that the extract was kept before injection was the same for all preparations, and the beginning of the injection period was so timed that it began the day following the completion of the preparation of the extract.

The yield of the oily extract (crude progestin) averaged 40 cc. per kilogram of corpora lutea for the 3 preparations. Considering 1 cc. of crude extract to be equivalent to 1 gm., this yield represents about 40 gm. per kilo of fresh tissue. Robson and Illingworth[3] reported having obtained about 30 cc. per kilo of corpora lutea, but have recently doubled this yield (personal communication).

The 2 extracts prepared from corpora lutea obtained from the local slaughter houses were administered to castrate adult female rabbits under the specified test conditions. An amount of extract equivalent to 70 gm. of fresh corpora lutea gave full proliferation (+++). Allen[1] found that generally one-half of the minimal dose necessary to produce +++ or ++++ proliferation will give a + or ++ reaction, and this was observed in the present study also, animals receiving 35 gm. equivalents of fresh tissue showing ++ reaction. Half of this dose, in turn, gave a very faint proliferation or no proliferation at all. From this it is seen that one rabbit unit was obtained from 70 gm. of fresh tissue and the yield of the active substance from extracts prepared in this way averaged 14 rabbit units per kilogram of fresh tissue.

When, however, an amount of the extract equivalent to 100 gm. of corpora lutea obtained from the Wilson Laboratories was injected ++ proliferation was observed. Half of this dosage gave a very faint reaction. These 2 results show a rather pronounced difference from those results given by preparations made from corpora lutea obtained from local sources, which were approximately twice as potent. This phenomenon has been observed repeatedly by Corner and Allen in their extensive work with pig material obtained from commercial laboratories and appears unexplainable in any way other than by the selection of material.

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[1] Allen, W. M., *Am. J. Physiol.*, 1930, **92**, 174.

[2] Corner, G. W., and Allen, W. M., *Am. J. Physiol.*, 1929, **88**, 326.

[3] Robson, J. M., and Illingworth, R. E., *Quart. J. Exp. Physiol.*, 1931, **21**, 93.

In this laboratory the general run of corpus luteum material obtained locally from sows gives a fairly constant yield of 30 to 50 rabbit units per kilogram of fresh tissue. The above results show that in the cow the corpora lutea contain much less progestin than do the pig's corpora lutea. As the same procedures were followed and the same laboratory was utilized, it seems probable that the lesser potency of cows' corpora lutea represents a species difference. The only other exact information on this point is that published by Robson and Illingworth,[3] whose results might be interpreted as indicating a higher potency of the cows' corpora lutea than that here reported; but from a very kind personal communication of Dr. Robson it appears that in his laboratory the yield measured by a test similar to ours is about 8 to 10 rabbit units per kilogram of fresh tissue, a figure close to our yield.

I wish to express my appreciation of the many helpful suggestions given by Dr. George W. Corner and Dr. Willard M. Allen.

8266 C

Effect of Sodium Amytal on Leucocytes of the Albino Rat.

DOUGLAS WARNER. (Introduced by F. G. Gilchrist.)

In Cooperation with the Department of Zoology, Pomona College, Claremont, Calif.

Male rats of Wistar Experimental Colony strain were used in a study of the effect of sodium amytal upon the white blood cell counts. Preceding and during the experiment, these animals were under standardized and controlled conditions as previously described.[1] The procedure was as follows: At 11, 12, and 13 (1 P.M.) o'clock wet counts for the total number of white cells per cc. of blood were made. At the same time smears were prepared for differential counts. Immediately after the 11 and 12 o'clock counts, saline solution was injected as a control. After the 13 o'clock count an aqueous solution of sodium amytal, 6 mg. per 100 gm. of body weight, was injected. Wet counts were made and smears prepared every 2 hours for the next 16 hours. Sixteen male rats were used in the experimental groups. The normal curve of 53 animals determined recently under identical conditions[1] was accepted as control.

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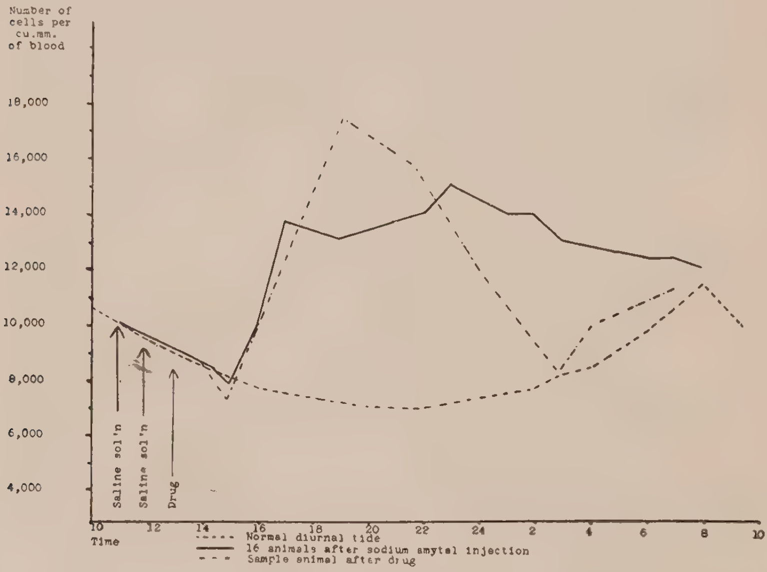
[1] To be published, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**.

The first 3 counts were used as a basis to determine the normal expectancy for each animal for each succeeding hour (based on the findings of previous work on the normal diurnal tide of the albino rat[1]). Let $X_1, X_2, - - - ,X_{16}$, represent the actual blood count of each of the 16 animals at any given hour. Let $Y_1, Y_2, - - - , Y_{16}$, represent the count normally expected for each animal for that hour had the drug not been administered. $X_1/Y_1, X_2/Y_2, - - - , X_{16}/Y_{16}$, then represent the ratio of actual to normal, or a measure of the effect of the drug at any time. Then $\frac{X_1/Y_1 + X_2/Y_2 + \cdots + X_{16}/Y_{16}}{16}$ equals the average measure of the effect, or gives a value by which the point on the normal curve must be multiplied in order to obtain the level of leucocytosis caused by the drug at a given hour.



A sample curve, plotted against the normal diurnal tide, is shown on the accompanying chart. The composite curve referred to above is found to be atypical of any individual but demonstrates a high degree of leucocytosis for the average of the group. The peak of leucocytosis may occur at any point from 5 to 11 hours after injection of the drug. This series of peaks accounts for the atypical composite curve. It was found that the increase in total count is caused by an excessive number of polymorphonuclear neutrophiles, which on the average increased to 144% of their normal expectancy. Lymphocytes dropped at first and then paralleled the normal at the level of about 75% of normal. Other cell types seemed to be unaffected.

These results were exactly opposite to the author's findings (unpublished) with amidopyrine and other cyclic drugs. Climenko[2] reports inhibition of leucogenic activity in the rabbit by certain cyclic compounds.

Conclusions. 1. The injection of a single dose of sodium amytal into albino rats caused a slight leukopenia 2 hours later, followed by a decided leucocytosis whose peak was reached at from 5 to 11 hours after injection. 2. The leucocytosis was caused by an increase in polymorphonuclear neutrophiles. 3. All counts returned to a level slightly above normal in from 11 to 19 hours.

8267 P

Oophorectomy and Maintenance of Pregnancy in the Rat.

H. O. HATERIUS.

From Washington Square College, New York University, and the Biological Laboratory, Cold Spring Harbor, L. I., N. Y.

It is generally agreed that removal of the ovaries during pregnancy, in the rat, invariably results in the untimely interruption of this condition.[1-4] Evidence is presented in this report, however, which demonstrates that under certain experimental conditions this rule does not hold, and that the rat may carry through to term during the latter part of pregnancy in the entire absence of ovarian tissue.

Adult female rats were subjected to unilateral resection of a Fallopian tube, to insure an initial litter size reduction. This was followed by exactly time mating. Laparotomy, performed on the 12th or 13th day of pregnancy, involved removal of one ovary and of foetuses in the fertile horn in excess of one. Foetal removal was so effected as to leave the placentae intact and *in situ.* Two days later in each instance the remaining ovary was ablated. Of 10 animals used in this series all carried to term, but failed to deliver, and the young were recovered alive after being allowed to remain *in utero* for one and 2 days past the expected date of parturition. Placentae, including those of previously removed foetuses, were viable and healthy.

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[2] Climenko, PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 823.

[1] Hain, A. M., *Q. J. Exp. Physiol.*, 1934, **24**, 101.

[2] Johnson, G. E., and Challans, J. C., *Anat. Rec.*, 1930, **39**, 195.

[3] Nelson, W. O., and Haterius, H. O., *Physiol. Zool.*, 1930, **3**, 300.

[4] Selye, H., Collip, J. B., and Thomson, D. L., *Endocrinol.*, 1935, **19**, 151.

A second group of 10 females was subjected to precisely the same treatment, with the exception that the 'extra' placentae were included in the surgical removal of foetuses on the 12th or 13th day. Following removal of the second ovary none carried to term. Autopsy on the expected date of delivery revealed markedly involuted uteri containing no indications of embryonic substance. A control group of 11 animals, treated in the same manner with the exception that the second ovary was not extirpated, carried through successfully, and normal delivery occurred in each instance at the expected time.

In the rat, therefore, the ovaries are not essential for the maintenance of pregnancy at least during the last third, providing (1) only one foetus is being carried, and (2) sufficient viable placental tissue is present. The birth mechanism, under conditions of the experiments, is inhibited.

These results, to be considered *in extenso* elsewhere, indicate that the placenta may supply progestin, and hence may serve in an auxiliary capacity to the ovaries. It is suggested that the degree of development of this auxiliary capacity may determine the relative freedom from dependence upon ovarian support encountered in certain mammals.

8268 C

Sex Reversal in Amblystoma. VIII. Sex Type of Gonads Developed from Gonadic Preprimordia of A. punctatum Implanted in Axolotl Females.*

R. R. HUMPHREY.

From the Department of Anatomy, University of Buffalo.

The writer[1] and Burns,[2] using different technical methods, have demonstrated that the testis (or potential testis) of *A. punctatum (maculatum)* may undergo reversal or transformation to an ovary when kept throughout development under the influence of a female of the more rapidly developing species, *A. tigrinum.* The structural changes involved in this reversal have been described.[1, 2] The present report offers further evidence of the occurrence of such reversal of testis to ovary.

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* Material obtained through the kindness of Dr. Cranford Hutchinson and the Morris Biological Farm of the Wistar Institute.

[1] Humphrey, R. R., *Anat. Rec.*, 1935, **62**, 223.

[2] Burns, R. K., Jr., *Anat. Rec.*, 1935, **63**, 101.

Right gonadic preprimordia of *A. mexicanum* (Mexican axolotl) embryos were removed and replaced by the corresponding preprimordia of *A. punctatum* donors. All operations were performed during tail-bud stages (28 to 32 of Harrison's series), employing the technique previously described.[3] Embryos serving as donors were reared whenever possible as a check upon the sex type of the gonad derived from the transplant. This gonad has been studied in serial sections in the case of hosts dying or killed during earlier stages, or its type has been ascertained by exploratory operation in the case of hosts surviving to more advanced ages (4 months or over). The sex of the host has been ascertained by similar study of its remaining gonad (on left side).

In the 37 hosts found to be females, the gonad derived from the transplant proved to be a testis in only 2 cases (5.4%) instead of the 50% expected on the basis of the approximately 1:1 sex ratio exhibited by the donor species. Of the donors furnishing transplants for these 37 hosts, 28 survived—16 females and 12 males. There are thus 10 cases in which the gonad of the transplant, although an ovary, is known to have been derived from a gonadic preprimordium supplied by a *male* donor. In 2 other cases the structure of the graft ovary indicates its probable development by reversal from a testis, since the cortex is relatively thin and the central cavity or ovarial sac contains free spermatogonia or other cells derived from the disintegrating testicular component of the gonad. Further, one of the 2 grafts classed as a testis, although it has attained a large size and has induced a pronounced modification of the ovary of the host (killed at 95 days), nevertheless exhibits features indicative of a probable later reversal to ovary (hypertrophy of the male cortex, marked degeneration of spermatogonia, and expansion of rete cords to form small ovarial sacs).

The findings noted above are in harmony with those previously reported by the writer[1] for *A. punctatum* transplants developing in *A. tigrinum* females; among the latter, normal testes were found in only 2 out of 88 cases. That these results cannot be dismissed as due to intermixture of host and graft tissues is shown by their close correspondence to the findings of Burns[2] in *A. punctatum* males joined in parabiosis to *A. tigrinum* females. Although in younger age groups the expected proportion of such pairs may be readily identified, pairs consisting of *punctatum* male and *tigrinum* female are almost completely lacking in older age groups, due to the tranformation of the testes of the *punctatum* cotwin into ovaries. In older grafted animals, as in older parabiotic twins, reversal of the testis is found to be completed, but in these the known male sex of the donor may afford conclusive evidence that reversal of the sex potentialities of the transplant has been induced by the female host.

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[3] Humphrey, R. R., *J. Exp. Zool.*, 1929, **53**, 171.

It is concluded that reversal of sex type in gonads derived from *A. punctatum* males is the general rule when these gonads develop in axolotl females, only an occasional graft testis proving capable of attaining a dominance over the ovary of the host and continuing its normal development.

8269 P

Pelvic Changes Occurring in Male Mice Receiving Large Amounts of Folliculin Benzoate.

W. U. GARDNER. (Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine.

The resorption of the pubic bones of the male pocket gopher has been induced experimentally by the administration of estrogenic hormone (Hisaw[1]). In the guinea pig 2 hormones, one of follicular origin and one present in the blood of pregnant rabbits or in the corpus luteum, were necessary to induce a relaxation of the pelvic ligaments (Hisaw[2]). In these species sexual dimorphism in the structure of the pelvis of matured animals exists. Males have the pubic bones well united by cartilage while the pubic bones of mature females have a ligamentous attachment.

This sexual dimorphism of the pubic symphysis was not noted in rats (Todd[3]). The pubic bones were well and firmly attached at the symphysis on both males and females. Observations to be reported here indicate that large amounts of estrogenic hormone are effective in inducing changes in the symphysis of the mouse in which a sexual dimorphism of the pelvis also occurs.

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[1] Hisaw, F. L., *J. Exp. Zool.*, 1925, **42**, 411.

[2] Hisaw, F. L., *Physiol. Zool.*, 1929, **2**, 59.

[3] Todd, T. W., *Am. J. Anat.*, 1923, **31**, 345.

Six male mice were given weekly subcutaneous injections of 500 international units of folliculin benzoate.* The injections were started at the time of weaning (28 days of age). The mice developed scrotal hernias after 6 to 8 weeks of treatment. One mouse was killed after 14 weeks of treatment and no change was noted in the pelvis. Five mice were removed after 19 to 28 weeks. At autopsy all presented greatly distended bladders, and 2 showed marked hydronephrosis and hydroureter. The urethras were greatly distended down to the region of the pubic symphysis. The region of the pubic symphysis was completely decalcified and ligamentous. The ligamentous band separated the pubic bones from 2 to 3 mm. The pelvis was easily movable as in the guinea pig at parturition. X-ray photographs of the pelvis verified the autopsy observations. The pubes, instead of coming to an apex at the symphyses, were widely separated. The symphyses of control male mice from 24 to over 200 days of age were broad and firmly united the pubic bones.

Two male mice of another strain (C_3H), which had received similar treatment from the time of birth for a period of 15 weeks also showed an absence of a pubic symphysis and the separation of the pubic bones by a ligamentous band. The testes did not descend and the scrota or hernias did not develop in these mice treated from birth. A similar separation of the pubic bones was observed in 4 other mice in the same series by palpation of the pelves.

It is suggested that the disarrangement of muscle attachments resulting from such changes in the pelvic architecture may influence the appearance of the scrotal hernias which have been previously observed by Burrows.[4] Also the factor causing retention of urine did not appear to be the prostatic hypertrophy induced as pointed out by Burrows,[5] as a constriction of the prostatic urethra has not been observed in mice with marked hydronephrosis and distended bladders, but due to a constriction of the urethra in the region of the pubic symphysis.

Further studies on these problems and on the nature of the pelvic change, whether decalcification and bone reabsorption or ligamentous proliferation, are being undertaken.

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* The folliculin benzoate was obtained from Professor Girard (Paris) through the courtesy of Dr. G. M. Smith. The preparation contained 10,000 international units of folliculin benzoate per cc. The mice used in this investigation were kindly supplied by Dr. L. C. Strong and were from two strains (A and C_3H) in his colony.

[4] Burrows, H., *Brit. J. Surg.*, 1934, **21**, 507.

[5] Burrows, H., *Am. J. Cancer*, 1935, **23**, 490.

8270 C

A Colorimetric Test for Guanidine.

M. X. SULLIVAN.

From the Chemo-Medical Research Institute, Georgetown University, Washington, D. C.

In studies pertaining to muscular dystrophies, attention was given to the possibility that simple guanidine derivatives might play a part in the etiology of these abnormalities. Guanidine is a protoplasmic poison as early shown by Gergens and Baumann[1] for animals and Kawahita[2] for plants. Just what part it might play in the phenomena of health and disease has been problematical since satisfactory tests for guanidine in body fluids, tissues, and excretions have been lacking. Various color tests have been recommended for guanidine by Tieg,[3] Marston,[4] and Weber.[5] These tests have not been found satisfactory since they give color more or less with methyl guanidine which may be formed from creatine or creatinine by oxidation as early shown by Dessaignes,[6] and corroborated by Ewins,[7] Baumann and Ingvaldsen,[8] and by Greenwald.[9]

Accordingly, a new test for guanidine $NH:C(NH_2)_2$ with a high degree of specificity was devised. This procedure runs as follows:

Color reaction for guanidine. To 1 cc. of an aqueous or decinormal hydrochloric acid solution of guanidine carbonate or hydrochloride, containing 0.5 mg. of guanidine base, add 1 cc. of a freshly made 1% solution of 1.2 naphthoquinone-4-sodium sulphonate followed by 0.3-0.5 cc. of N NaOH. Let stand 15 minutes at 20°C. or, better, 1-2 minutes in a boiling water bath. Cool under running tap water, add 0.3 cc. of a 25% urea solution and acidify with 1 cc. of concentrated HCl, mix, and add 1 cc. of concentrated HNO_3 (density 1.42). The guanidine solution becomes a bright red while other compounds tested, with a few exceptions later detailed, go to yellow. The guanidine reaction is definitely red with solutions containing 0.05 mg. guanidine per cc. With 0.5-1 mg. of guanidine per cc. a brown red precipitate is formed, soluble in alcohol. In such cases add a few cc. of alcohol to standard and unknown.

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[1] Gergens, E., and Baumann, E., *Pflügers Arch. f. Physiol.*, 1876, **12**, 265.

[2] Kawahita, I., *Bull. Coll. Agr. Tokyo*, 1908, **6**, 161.

[3] Tieg, I. W., *Australian J. Exp. Biol. Med. Sci.*, 1924, **1**, 93.

[4] Marston, H. R., *Australian J. Exp. Biol. Med. Sci.*, 1924, **1**, 99.

[5] Weber, C. J., *J. Biol. Chem.*, 1928, **78**, 465.

[6] Dessaignes, M., *Compt. rend.*, 1854, **38**, 839.

[7] Ewins, A. J., *Biochem. J.*, 1916, **10**, 103.

[8] Baumann, L., and Ingvaldsen, T., *J. Biol. Chem.*, 1918, **35**, 277.

[9] Greenwald, I., *J. Am. Chem. Soc.*, 1919, **41**, 1109.

In estimating guanidine in mixtures of amino acids more naphthoquinone must be added. Two cc. of the 1% aqueous solution of 1.2 naphthoquinone-4-sodium sulphonate have been found ample in all cases detailed in this paper.

The urea is added to lessen fading of the red color. If methyl guanidine is present in appreciable amounts, and only nitric acid is used, the final solution starts yellow but gradually develops a brown color. If concentrated hydrochloric acid is added before the nitric acid, methyl guanidine gives no red color. If no methyl guanidine is present the addition of hydrochloric acid before the nitric can be dispensed with.

In this procedure the color is red with guanidine, guanidine glyoxylic acid, and guanidine oxalic acid (which readily split off the glyoxylic acid and oxalic acid), yellow with methyl guanidine, amino guanidine (which starts red but fades quickly to yellow), glycocyamine, glycocyamidine, creatine, creatinine, hydantoic acid, arginine, biuret, xanthine, methyl xanthine, adenine, guanine, uric acid, allantoin, alloxan, glycine, alanine, phenylalanine, aspartic acid, valine, cysteine, cystine, glutamic acid, leucine, proline, tryptophane, histidine, histamine, lysine, thymine, uracil, urea, thiourea, acetone, pyruvic acid, catechol, adrenaline, dioxyphenylalanine, hydrazine, dextrose, parabanic acid, urethane, etc., each 1 mg. per cc. in solution or suspended. The only positive interferers are large amounts of ammonia or methylamine, indol, and benzidine.

Benzidine can be easily eliminated by precipitation as a sulphate and filtering. Indol is readily volatilized by heating in a slightly acid solution. Thus 1 cc. each of 2 indol solutions, one containing 1 mg. of indol in 95% alcohol and the other a saturated aqueous solution, treated with 0.2 cc. of N HCl and heated on the water bath for 10 minutes, before making the guanidine test, were negative.

In biological media, methyl amine and ammonia will rarely if ever be met with in amounts sufficient to interfere with the guanidine test. If such amounts are present, the interference can be eliminated by precipitating the guanidine with picric acid as done in the reaction dealing with urine. Guanidine readily forms an insoluble picrate which gives the guanidine reaction, while the picrates of methyl amine and ammonia are readily soluble.

In a mixture containing 10 mg. each of tyrosine, phenylalanine, dioxyphenylalanine, cystine, leucine, alanine, glycine, valine, proline, aspartic acid, glutamic acid, arginine (as carbonate), lysine, histidine, tryptophane, creatinine, creatine and 100 mg. of guanidine (as guanidine hydrochloride, 161.7 mg. in 200 cc. of 0.1 N HCl), guanidine can be matched quantitatively against the same quantity of guanidine in 0.1 N HCl.

In this case, however, more of the naphthoquinone must be used because of the large content of amino acids. The following procedure gave practically quantitative results: (A) To 1 cc. of the guanidine amino acid mixture add 2 cc. of 1% aqueous solution of 1.2 naphthoquinone-4-sodium sulphonate, mix and add 0.5 cc. of N NaOH, heat in water bath for 1 minute, cool, and add successively with mixing 0.5 cc. of 25% aqueous urea solution, then 0.5 cc. of concentrated HCl and 1 cc. of concentrated HNO_3 and finally 5 cc. of 95% alcohol to put precipitate into solution. (B) One cc. of guanidine (0.5 mg. per cc.) in 0.1 N HCl similarly treated. (B) set at 20, (A) reads 20.7.

Guanidine added to urine. The quantitative determination of guanidine added to urine offers difficulties. In some urines the detectable guanidine speedily disappears, due apparently to tying with urinary ingredients. In a number of cases guanidine added to urine has been estimated with 90-100% accuracy shortly (within an hour) after the addition to the urine. Even in these cases the urine requires special treatment before applying the color reaction, the details of which are somewhat outside the scope of the present paper. Aside from the addition of guanidine to urine no unsubstituted guanidine has been found in any of the normal or pathological urines examined. As will be shown, however, by Sullivan, Hess, and Irreverre, in progressive muscular dystrophy and especially in pseudohypertrophic muscular dystrophy, there occur in the urine simple guanidine derivatives which by mild oxidation yield guanidine. Such guanidine derivatives have not been found in normal urine in any appreciable amounts nor in any other pathological urine so far investigated.

8271 P

Detection of Ovulation in the Intact Rabbit.*

H. S. BURR, R. T. HILL, AND EDGAR ALLEN.

From the Yale University School of Medicine, New Haven, Conn.

The determination of the electrical characteristics of living systems has been severely limited by the nature of the measuring devices available. During the past year and a half, one of us (H.S. Burr), with the help of C. T. Lane of the Physics Laboratory, has developed a vacuum tube potentiometer which is stable, draws almost no current from the living system and is independent of inter-electrode resistance up to one megohm. With this instrument, it is possible to record accurately minute voltage changes in living systems under a wide variety of circumstances. Furthermore, with the aid of L. F. Nims of the Department of Physiology, reproducible silver-silver chloride electrodes have been made, by means of which many artefacts have been eliminated.

Students of reproduction have long been searching for some external sign of ovulation in order to accurately place this event in the time sequences of the oestrous and menstrual cycles, and for use in dating and controlling conception. Ovulation was not actually observed until 1928. Walton and Hammond[1] first described this process in the rabbit. This animal was chosen for it has long been known that the rabbit ovulates about 10 hours after mating. To extend and reexamine the observations of Walton and Hammond, a moving picture of ovulation in the rabbit has just been completed.[2] This film showed the process of ovulation to be truly explosive in nature and led us to believe that such a rupture should be accompanied by electrical changes of sufficient magnitude to be measurable with the above potentiometer.

Putting this to test, it has been possible to read differences in electrical potential in the following way. Rabbits were mated and approximately 6 hours later were surgically anesthetized with sodium amytal. The abdominal hair was shaved and the animal placed on an operating board. A salt bridge leading to a silver-silver chloride electrode was placed on the abdomen. Another salt bridge led from a glass cannula in the vagina to another silver-silver

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* Aided by a grant from the Research Funds of the Yale University School of Medicine.

[1] Walton, A., and Hammond, J., *Brit. Exp. Biol.*, 1928, **6**, 190.

[2] Hill, R. T., Allen, Edgar, and Kramer, T. C., *Anat. Record*, 1935. In press.

chloride electrode. Leads from the 2 electrodes were connected to the instrument. Then observations were made of changes in electromotive force preceding and during the known period of ovulation.

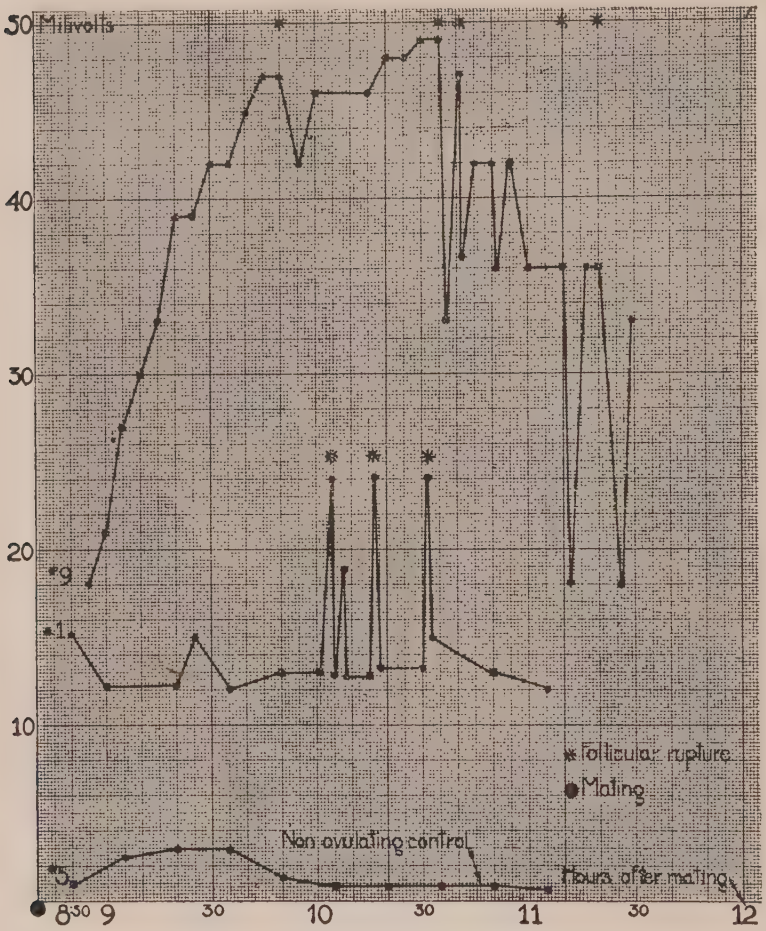

Fig. 1.

Voltage changes in the intact rabbit during follicular rupture.

Readings taken previous to the eighth hour after mating established a fairly uniform base line. Subsequently, a distinct but gradual rise from the base line occurred. In the first rabbit, as the ninth hour after mating approached, a sharp rise of potential occurred which was 4 to 5 times greater than the control variation. Twelve minutes later a similar change was repeated. A third rapid rise and fall occurred 17 minutes after the second surge. Following these records examination of the ovaries at laparotomy showed 3 rupture points, 2 in one ovary and one in the other. In rabbit No. 3, during a 5 hour period, 5 rises in electrical potential were recorded and 5 ruptured follicles were found in the ovaries. In rabbit No. 6, observed for 3½ hours, one peak was recorded and a single follicle had ruptured.

The increase in voltages recorded during ovulation have varied from 7,000 to 20,000 microvolts; variations in the non-ovulating control were from 500 to 2500 microvolts.

In order to determine the exact relationship between the voltage surges and follicular rupture, 3 attempts have been made to record the electrical changes while the ovary was under direct observation. The first 2 attempts failed because the rabbits did not ovulate while under observation and no voltage surges appeared. The third attempt (rabbit No. 9) was successful. Distinct but gradual rises from the base line occurred coincident with observed enlargement of the follicles during the hour preceding the first rupture. Usually sharper rises were noted during the final rapid distension just before rupture. Immediately following the rupture of the follicles, the voltage dropped to the vicinity of the base line.

Five non-ovulating control rabbits observed from one to 11 hours failed to show these sharp rises. In all animals observed to date there has been an exact correlation between the number of surges in electrical potential and the number of follicles ovulated.

These findings suggest that with the aid of this new instrument it is possible to determine with great certainty in the intact animal the time and duration of ovulation, the instant of follicular rupture and the exact number of ovulations. These studies are now being extended to other animals, including primates, and to an analysis of the factors responsible for the voltage changes.

8272 P

Effect of Reduced Oxygen Tension Upon Formed Elements of Blood of Hypophysectomized Animals.*

GERTRUDE E. STEWART, ROY O. GREEP, AND OVID O. MEYER.

From the Departments of Medicine and Biology, University of Wisconsin.

It has been suggested[1, 2] that the hypophysis, in addition to its many other functions, may play a part in erythropoiesis.

It has long been known that in man and animal, under the conditions of reduced oxygen tension, there occurs an increase in hemoglobin, erythrocytes and reticulocytes in the blood, and hyperplasia of the bone marrow. It was thought that if the hypophysis furnished a hormone which affected the erythrogenetic tissues, the response to a diminution in oxygen tension in the tissues of hypophysectomized animals might be deficient in one or more respects. To test this hypothesis, 8 male white rats weighing 150 to 220 gm. were hypophysectomized after complete blood counts were done by the usual methods. Erythrocyte counts were done in duplicate; reticulocyte counts of 1,000 cells on a cover slip smear stained with cresyl blue, hemoglobin determinations with the Haden-Hauser hemoglobinometer. Eight days later, 4 of the experimental animals with 4 normal controls of similar weight were placed in the respiratory chamber devised by Dallwig, Kolls and Loevenhart.[3] The remaining 4 animals were placed in the chamber 25 days after hypophysectomy, and a second group of 8 hypophysectomized animals was similarly divided and exposed to the same experimental procedures 9 and 32 days after operation. In all instances complete blood counts were done on the day the animals were placed in the chamber. The pressure was reduced in all experiments to 422 mm. Hg.; equivalent oxygen tension, 12% of an atmosphere, which corresponds to an altitude of approximately 16,000 feet. This level was maintained constantly with but 2 to 3 mm. Hg. variation. Blood counts were made at intervals between 3 and 6 days. Some of the animals died in the first week under the experimental conditions, so that adequate data were obtained on but 14 hypophysectomized and 14 normal animals. The results were remarkably constant and they appear to be significant.

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* This study was in part supported by a grant from the Wisconsin Alumni Research Foundation.

[1] Evans, H. M., *J. A. M. A.*, 1935, **104**, 464.

[2] Moehlig, R. C., and Bates, G. S., *Arch. Int. Med.*, 1933, **51**, 207.

[3] Dallwig, H. C., Kolls, A. C., and Loevenhart, A. S., *Am. J. Phys.*, 1915, **39**, 67.

The 14 control rats, in the chamber for periods of 3 to 12 days, showed an average increase of hemoglobin from 14.6 to 16.7 gm. Simultaneously, the erythrocytes increased 700,000 and the reticulocytes from a control level of 2% to a peak of 7%. There was no significant change in the weight of these animals during the experiment.

The 7 rats that were exposed to the reduced pressure 8 and 9 days after hypophysectomy, exhibited a similar rise in the hemoglobin and erythrocytes. The hemoglobin rose from a level of 14.6 to 16.8 gm. and there was an average increase of 1,300,000 erythrocytes. The reticulocytes, however, which fell from a preoperative level of 2.9% to 0.1% after hypophysectomy, showed no response to the stimulus of oxygen want, and the highest level was 0.2%. The average weight loss of these animals, while in the chamber was 23 gm.

The 7 rats that had had hypophysectomy 25 and 32 days before being placed in the chamber, however, showed no significant rise in the hemoglobin or erythrocyte count. The control level was 13.2 gm., at the end of the experiment the hemoglobin was 13.5 gm. The control erythrocyte count was 8,410,000, the final count 8,545,000. The reticulocytes, which averaged 3.3% before operation, fell to 0.3% the day the experiments started. At the conclusion of the experiments they numbered 0.1%. The average weight loss of these animals while under the influence of the reduced pressure was 13 gm.

Most striking of these results is the sharp fall in the number of reticulocytes after hypophysectomy and the failure of increase under the stimulus of oxygen want. In the experiments done 8 and 9 days after hypophysectomy there was a regular increase in hemoglobin and erythrocytes simulating in degree that of the controls, whereas in the experiments done 25 and 32 days after hypophysectomy there was just as regularly no significant increase in these components of the blood. If a specific hypophyseal hormone exists, it might be argued that some residue persisted in the tissues for a short time after hypophysectomy but was absent after a longer postoperative period. These results may not be due to a specific hormone; the action may be an indirect one upon some other organ, or may be due to the general derangement in metabolism that follows hypophysectomy.

Examination of the bone marrow of several of these hypophysectomized animals has been made. This shows a lack of the pronounced hyperplasia noted in the marrow of the controls; thus the marrow resembles that of normal animals at atmospheric pressure.

A conclusion as to the significance of these results is not as yet possible but they seem to offer several interesting possibilities. Further studies under similar conditions with the removal of other endocrine glands are being done.

While these studies were in progress the interesting reports of Dodds and his associates[4, 5] were published. They have noted the development of an anemia and a marked reticulocytosis in association with gastric lesions upon the injection of pituitary extract. They conclude that a hormonal connection may possibly exist between the posterior lobe of the pituitary gland, the stomach, and the blood.

8273 P

Effect of Vitamins B and G Deficient Ration on a Coccidian Infection.

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From Iowa State College.

Fifteen half-grown rats were put on the following diet, in order to develop in them the early symptoms of vitamins B and G deficiency: extracted casein, 18 pts.; starch, 73 pts.; butter, 4 pts.; salt mixture, 4 pts.; cod liver oil, 1 pt. The 15 controls received the same mixture made up to 8.0% with Fleischmann's powdered yeast. After 9 days on these rations each rat was infected *per os* with 1500 sporulated oocysts of *Eimeria miyairii* daily for 5 successive days. The oocysts eliminated during the infections were collected during the patent period in pans beneath the wire cages, and counts were made by the counting and dilution method.

The rats on the deficient diet eliminated from 2 million to 91 million oocysts each; mean, 35.8 ± 4.5 million. The controls on the normal diet eliminated from 106 million to 275 million oocysts each; mean 167.27 ± 7.15 million. It appears that the deficient rats developed infections only from a fourth to a fifth as heavy as the control rats. Both series were refractory to subsequent reinfection when restored to the growing diet (Steenbock's).

No explanation is offered at present, but it appears that the yeast contains something that favors the development of a coccidian infection. Work is now in progress to separate the influence of vitamins B and G.

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[4] Dodds, E. C., Hills, G. M., Noble, R. L., and Williams, P. C., *Lancet*, 1935, 1099.

[5] Dodds, E. C., and Noble, R. L., *Nature*, 1935, **135**, 788.

New York Meeting

New York Academy of Medicine, October 16, 1935.

8274 C

Metabolism of Bromobenzene in Growing Dogs and Mice Maintained on Adequate Diets.*

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Monohalogen benzenes, when fed, are excreted in the urine of adult dogs,[1] cats,[2] rats,[3] and rabbits,[4] partly as ethereal sulfates, partly as mercapturic acids. Hele,[5] in an attempt to compare the synthesis of mercapturic acid in dogs and pigs, has come to the conclusion that the pig does not synthesize mercapturic acid readily. Hele[5] used young growing pigs as experimental animals in comparing the metabolism of bromobenzene in pigs to that in the adult dog. Inasmuch as Abderhalden[6] believes that the synthesis of mercapturic acid is limited in the animal body by the need of that animal for sulfur for reactions more essential than the detoxication of bromobenzene, it seemed probable that the limitation of synthesis of mercapturic acid in the pig as found by Hele[5] was due to the fact that he employed young growing pigs instead of the adult animal. White and Jackson[7] have adduced evidence which seems to indicate that bromobenzene, when fed to growing rats, affects the utilization by the animals of cystine necessary for normal growth. These workers have not, however, demonstrated that mercapturic acid was actually synthesized by the growing rat.

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* I wish to express my gratitude to Dr. F. Brogan and to Mr. A. Vajda for the analysis of the mercapturic acid.

[1] Baumann, E., and Preusse, C., *Ber. deutsch. chem. Ges.*, 1879, **12**, 806.

[2] Gibson, R. B., *J. Biol. Chem.*, 1909, **6**, 16.

[3] Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037.

[4] Abderhalden, E., and Wertheimer, E., *Pflüger's Arch.*, 1925, **207**, 215; **209**, 611; Nishimura, K., *Acta schol. med. univ. imp. Kioto*, 1929-30, **12**, 73; Lawrie, N. R., *Biochem. J.*, 1931, **25**, 1037; Lough, S. A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **94**, 739.

[5] Coombs, H. I., and Hele, T. S., *Biochem. J.*, 1927, **21**, 611.

[6] Abderhalden, E., and Wertheimer, E., *Z. physiol. Chem.*, 1931, **198**, 8; **201**, 267.

[7] White, A., and Jackson, R. W., *J. Biol. Chem.*, 1933, **100**, ciii.

As far as we know, the metabolism of bromobenzene in the growing dog or growing white mouse has not been studied previously. Pending the development of the method for the estimation of mercapturic acids in the urine, now in progress, we used the changes in the partition of urinary sulfur produced by bromobenzene feeding to animals, and the isolation of mercapturic acid from the urine as the criterion of the synthesis of mercapturic acid by the growing dog and mouse, while maintained on adequate diets.[8] It was anticipated that the metabolism of bromobenzene in the growing dog would be similar to that in the adult animal, inasmuch as the metabolism of naphthalene, which also yields mercapturic acid, was found by us to be the same in the adult and growing dog.[9]

Three female mongrel pups of 2 to 7 months of age and one Dalmatian male thoroughbred pup of 5 months were used. The general experimental procedure and the diet used were the same as used previously.[9] Fifteen 21-days-old white mice, born and raised in the laboratory were fed *ad libitum* a diet consisting of 70 parts of oats, 15 parts of milk powder and 15 parts of yeast powder (Harris) and placed in a group in a small false-bottom metabolism cage. The cage was placed over a large funnel which contained a small filter to separate the feces from the urine. 0.1 cc. of bromobenzene was injected into each mouse subcutaneously on 2 consecutive days and the urine collected. No attempt was made at this time to secure any analytical data similar to that obtained on the growing dog's urine, in view of the obvious impossibility to maintain mice in equilibrium. All animals received water *ad libitum*.

p-brom-phenylmercapturic acid was isolated by the method of McGuinn and Sherwin.[10] The isolations were made on the 24-hour sample of each pup separately and on the entire 2-day sample of urine collected from the fifteen mice.

Table I shows the results obtained on the pups. The data apparently indicate formation of an ethereal sulfate and a sulfur compound or compounds which are excreted in the neutral sulfur fraction of the urine. The raised excretion of neutral sulfur continued for 2-3 days after the feeding of bromobenzene. It was found that

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[8] Stekol, J. A., *J. Biol. Chem.*, 1934, **107**, 641; 1935, **109**, 147.

[9] Stekol, J. A., *J. Biol. Chem.*, 1935, **110**, 463.

[10] McGuinn, A., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1115.

TABLE I.
Metabolism of Bromobenzene in Growing Dogs Maintained on Cowgill's Diet.

	Intake			Urinary Output						
	Age	N	S	Total N	Urea N	Total S	Total SO_4S	Inorganic SO_4S	Ethereal SO_4S	Neutral S
	days	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Pup 21 ♀	58	3.0	.155	2.42	1.72	.104	.055	.043	.012	.049
3.15 kg.	59	”	”	2.26	1.80	.095	.052	.040	.012	.043
	60	”	”	2.91	2.30	.160	.071	.025	.046	.089*
	61	”	”	3.29	2.60	.087	.017	.000	.017	.070
	62	”	”	2.52	1.95	.090	.049	.036	.013	.041
	63	”	”	2.37	1.93	.090	.046	.030	.016	.048
Pup 21 ♀	126	4.8	.248	3.29	2.39	.173	.121	.103	.018	.052
5.0 kg.	127	”	”	3.26	2.37	.144	.100	.083	.017	.044
	128	”	”	3.36	2.48	.195	.088	.044	.044	.107*
	129	”	”	3.54	2.87	.096	.019	.001	.018	.077
	130	”	”	3.44	2.50	.139	.085	.068	.017	.054
	131	”	”	3.32	2.53	.148	.102	.086	.016	.046
Pup 22 ♀	56	3.0	.155	2.02	1.45	.079	.042	.032	.010	.037
2.72 kg.	57	”	”	2.16	1.61	.091	.052	.041	.011	.039
	58	”	”	2.60	1.82	.151	.053	.023	.030	.098*
	59	”	”	2.81	2.17	.077	.016	.000	.016	.061
	60	”	”	2.10	1.46	.081	.040	.027	.013	.041
	61	”	”	2.00	1.40	.073	.041	.030	.011	.032
Pup 18 ♂	140	6.6	.341	4.56	3.88	.225	.149	.128	.021	.076
Dalmatian	141	”	”	4.35	3.53	.212	.140	.120	.020	.072
7.0 kg.	142	”	”	4.51	3.75	.233	.150	.129	.021	.083
	143	”	”	4.65	4.02	.285	.121	.080	.041	.164*
	144	”	”	5.06	4.26	.158	.037	.012	.025	.121
	145	”	”	4.50	3.74	.234	.128	.110	.018	.106
	146	”	”	4.81	3.78	.253	.144	.123	.021	.109
	147	”	”	4.89	3.70	.220	.134	.112	.022	.086
Pup 19 ♀	190	6.0	.310	4.43	3.00	.169	.129	.109	.020	.040
6.0 kg.	191	”	”	4.33	2.92	.160	.120	.100	.020	.040
	192	”	”	4.40	3.00	.180	.077	.046	.031	.103*
	193	”	”	4.70	3.40	.161	.105	.080	.025	.056
	194	”	”	4.40	3.10	.174	.117	.098	.019	.057
	195	”	”	4.31	3.00	.175	.120	.100	.020	.055

*1.0 gm p-Bromobenzene fed in gelatin capsule at 11 a.m.

as long as the neutral sulfur excretion continued above normal values, mercapturic acid was present in the urine. The raised output of neutral sulfur of the urine extending over a period of 2-3 days may indicate either slow synthesis of mercapturic acid in the growing dog or delayed excretion of the acid from the body or both. Analysis of the purified p-bromo-phenylmercapturic acid gave the following data: M.P. 152°C. (uncorr.); N, 4.21%; S, 10.39%. (Calculated for Br $C_{11}H_{12}O_3N$ S, N, 4.40; S, 10.08.) The average yield of purified p-brom-phenylmercapturic acid from each 1.0 gm. dose of bromobenzene fed to pups was 120-130 mg.

p-brom-phenylmercapturic acid isolated by a similar method from the urine of mice gave the following data: M.P. 151-152°C. (uncorr.) N, 4.27%; S, 9.97%.

Neither creatinine, uric acid nor allantoin output in the urine of pups were affected by the bromobenzene feeding. Naphthoresorcinol test for glycuronates was positive on the urine of all pups which were fed bromobenzene. No toxic effects were noticeable in the growing dogs after the administration of 1.0 gm. bromobenzene and the animals appeared quite normal. The mice, however, showed unmistakable signs of intoxication such as depression, loss of vitality and even complete prostration, resulting, in over 50% of the cases, in death.

The data presented indicate that the growing dog and mouse, under the conditions employed, are able to synthesize p-brom-phenyl mercapturic acid from the administered bromobenzene. The partition of various nitrogenous and sulfur fractions of the urine of growing dogs on the day of administration of bromobenzene is essentially similar to the partition of the same constituents in the urine of adult dogs which were fed bromobenzene while maintained on adequate diets.[11] The need of the growing dog for sulfur of the food did not prevent the animal from synthesizing the mercapturic acid from bromobenzene. We thought it possible that the synthesis of mercapturic acid by the growing dog took place because of the presence of cystine and methionine in the diet in excess to the needs of the animal for growth and maintenance. We found, however, in experiments which will be published shortly, that neither the adult dog nor the growing dog, while maintained on low-sulfur diets, protein-free diets, or after a fast of 4-5 weeks' duration was prevented from synthesizing mercapturic acid from bromobenzene. While it is probable that the inability to synthesize mercapturic acid by the growing pig is a species characteristic, experiments of Shiple, Muldoon and Sherwin[12] indicate the synthesis of mercapturic acid by the adult pig, if we accept the rise in the neutral sulfur of the urine on the day of feeding bromobenzene as a criterion of the synthesis of mercapturic acid, as has been done by Hele[5] and White and Lewis.[11] Since it has been shown by McGuinn and Sherwin[10] that the conventional methods for the isolation of mercapturic acid from the urine are not reliable, the failure of Hele[5] to isolate mercapturic acid from the urine of the pig may possibly be due to the unreliability of the Baumann and Preusse[1] method and not to the absence of mercapturic acid in the urine.

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[11] White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

[12] Shiple, G., Muldoon, J., and Sherwin, C. P., *J. Biol. Chem.*, 1924, **60**, 59.

Summary. 1. Bromobenzene was fed to mongrel pups, to a Dalmatian Coach pup and injected into growing mice. 2. p-bromphenylmercapturic acid was isolated from the urine of all pups studied and from the urine of growing mice.

7275 C

Synthesis of p-Brom-phenylmercapturic Acid by the Fasting Growing Dog.

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The literature on the subject of mercapturic acid synthesis in animals under various dietary regimes has been reviewed by White and Lewis.[1] One group of workers is of the opinion that exogenous cystine sulfur alone determines the synthesis of mercapturic acid,[2] while others maintain that the body tissues are capable of supplying cystine to meet the needs for detoxication of administered brombenzene.[3] McGuinn and Sherwin,[4] contrary to Abderhalden,[3] have demonstrated that acidity or alkalinity of the diet has no effect on the synthesis of phenyl-mercapturic acid in rabbits. By chloroform extraction method, McGuinn and Sherwin[4] were able to isolate mercapturic acid from the urine of rabbits fed brom-benzene while maintained on either acid or alkaline diets.

Thomas[2] was unable to detect mercapturic acid in the urine of fasting dogs. Abderhalden,[3] however, isolated mercapturic acid from fasting dogs' urine, but he believes that the cystine requirements for more essential functions of the body limit the degree of mercapturic acid synthesis.

In all of the above mentioned experiments on fasting dogs, including that of Nishimura's on fasting rabbits,[3] the period of fasting was rather short. The experiments did not exclude the possibility of liberation of cystine from the so-called circulating or stored protein which, as has been shown long ago,[5] is not depleted after short periods of complete fasting. Ashworth and Brody[6] have further shown "that the previous level of dietary protein has a marked influence on the time necessary to reach the endogenous level of nitrogen excretion in the rat."

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[1] White, A., and Lewis, H. B., *J. Biol. Chem.*, 1932, **98**, 607.

[2] Thomas, K., and Straczewski, H., *Arch. Anat. u. Physiol., Physiol. Abt.*, 1919, 249; Kapfhammer, J., *Z. physiol. Chem.*, 1921, **116**, 302; Muldoon, J. A., Shiple, G. J., and Sherwin, C. P., *J. Biol.*, 1924, **59**, 675.

[3] Abderhalden, E., and Wertheimer, E., *Z. physiol. Chem.*, 1931, **198**, 18; **201**, 267; Nishimura, K., *Acta Schol. med. univ. imp. Kioto*, 1929-30, **12**, 73.

[4] McGuinn, A., and Sherwin, C. P., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1115.

Experiments were undertaken in order to test the ability of the animal to synthesize mercapturic acid after a prolonged fast of at least 4-5 weeks. It seemed of interest also to ascertain whether a growing animal is capable of furnishing cystine for detoxication of brom-benzene in spite of the need of the animal for the essential factor and in spite of the prolonged fast.

Animals raised in this laboratory on Cowgill's synthetic diet[7] were used. The dogs were confined to individual metabolism cages. At the age of 5-6 months the food was withdrawn and the animals were fasted for 26-35 days. Water was allowed *ad libitum*.

"Adolph," a male pup of 5.9 kilos of weight, was given 1.0 gm. of brom-benzene in a gelatin capsule on the 26th day of fast and the urine was collected on the days following the feeding, every 24 hours. On the 28th day of fast, a similar dose of brom-benzene was given again. Phenyl-mercapturic acid was isolated directly from the urine on the day of collection by the method of McGuinn and Sherwin.[4] We were able to isolate 35 mg. of mercapturic acid from the urine for each gram of brom-benzene fed. The analysis of the product gave the following results:

Found:	Theory:
M.P. 152-153°C. (uncorrected)	
S—10.2%	S—10.08%
N— 4.46%	N— 4.40%

The isolated product gave a positive thiophenol reaction with concentrated sulfuric acid.

Similar results were obtained with "Isolda," a female pup which was fasted for 35 days.

The data on the distribution of various nitrogenous and sulfur constituents of the urine of fasting dogs which were fed brom-benzene are not presented here because no reliable information as to the meaning of the fluctuations in the values, especially of the neutral sulfur, can be derived therefrom. The animals showed unmistakable symptoms of intoxication on the days of feeding of brom-benzene, such as depression, loss of vitality and even prostration.

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[5] Voit, C., *Z. f. Biol.*, 1866, **2**, 307; Voit, C., L. Hermann's "Physiologie," Leipzig, 1881, 6, Pt. 1, p. 300, as quoted by G. Lusk, Science of Nutrition, 4th edition, p. 80.

[6] Ashworth, U. S., and Brody, S., Missouri Res. Bul. 190, 1933.

[7] Cowgill, G. R., Deuel, H. J., Jr., and Smith, H. H., *Am. J. Physiol.*, 1925, **73**, 106.

The isolation of phenylmercapturic acid from the urine of fasting growing dogs seems to indicate that the body tissues are capable of supplying cysteine for the detoxication of brom-benzene, even under such drastic conditions as employed by us.

Summary. 1. Brom-benzene was fed to growing dogs which were fasted for 4-5 weeks prior to the administration of brom-benzene. 2. Mercapturic acid was isolated from the urine of pups on the day following the feeding of brom-benzene and identified by analysis. 3. Fasting growing dogs apparently are capable of supplying cysteine for detoxication purposes at the expense of tissue.

8276 C

Effect of Anterior Hypophysis Emulsion on Natural Resistance of Hypophysectomized and Normal Rats to Histamine Poisoning.

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In previous work we studied the effect of partial and complete hypophysectomy in rats on the natural resistance to histamine poisoning and correlated the anatomical changes in the suprarenal gland with the variations in resistance.[1] We noted that the natural resistance of completely hypophysectomized rats to histamine poisoning was depressed during a period of one to ten weeks after operation. The M.L.D. was one-fifth to one-third that for normal rats. This decrease in resistance, we found, was associated with involutional changes of the suprarenal gland, such as hemorrhage into or atrophy of the inner zones of the cortex.[2] Rats in which the posterior lobe and a large portion of the anterior lobe were removed showed a similar drop in resistance and atrophic changes in the suprarenal cortex occurred, but where a large fragment of the anterior lobe remained no depression in resistance to histamine occurred and the suprarenal glands were normal. We concluded that the drop in natural resistance following hypophysectomy in the rat was probably secondary to the atrophic changes of the suprarenal cortex induced by the withdrawal of the adrenotropic hormone of the anterior lobe.

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[1] Perla, D., and Rosen, S. H., *Arch. Path.* In press.

[2] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 655.

In subsequent studies it was found that repeated injections of large amounts of suprarenal cortical hormone raised the natural resistance of totally hypophysectomized adult rats to histamine poisoning. In some instances the resistance was raised almost to the level of normal rats. This was particularly marked during the first 2 weeks after operation, the period during which the most severe depression in natural resistance occurs. The M.L.D. of histamine for hypophysectomized rats within 2 weeks after operation varied from 200 to 300 mg. per kg. of body weight. Hypophysectomized rats treated with cortical hormone for 6 days prior to the injection of histamine survived, in many instances, 800 mg. of histamine per kg. of body weight.

These observations tended to support the hypothesis that the depression in natural resistance following hypophysectomy was due to the impairment of the function of the suprarenal cortex resulting from the withdrawal of the adrenotropic hormone of the anterior lobe.

In the present communication the effect of repeated injections of a crude emulsion of the anterior lobe of the hypophysis of the ox on the resistance of hypophysectomized and normal rats to subsequent injections with histamine was studied.

All the rats used in these experiments were 3 to 4 months old and of a uniform stock raised in our laboratory for many years. Twenty-eight rats were hypophysectomized, then divided into 2 groups. Nineteen were treated daily with 1 cc. of crude saline emulsion of fresh ox anterior hypophysis; 9 were untreated. At the end of 14 days all were injected with varying amounts of histamine. Those rats treated with emulsion were given histamine in amounts ranging from 400 to 800 mg. per kg. of body weight. Those that were not treated were injected with histamine in amounts ranging from 200 to 600 mg. per kg. of body weight. Three normal controls received 900 and 1000 mg. of histamine per kg. It will be seen from Table I that the natural resistance of hypophysectomized rats treated with anterior hypophyseal emulsion to histamine poisoning was raised considerably. These rats gained greatly in weight and atrophy of the glands of internal secretion and visceral organs did not occur. In many instances the rats survived 700 to 800 mg. of histamine per kg. of body weight. The M.L.D. for untreated hypophysectomized young adult rats varied from 200 to 300 mg. of

TABLE I.
Effect of Repeated Injections of Anterior Hypophyseal Saline Emulsion on the Natural Resistance of Hypophysectomized Albino Rats to Histamine Poisoning (Ergamine Acid Phosphate).

No. Rats	Amt. histamine per kg.	Survived	Died
Hypophysectomized rats treated with anterior hypophyseal emulsion.*			
2	400	2	0
7	600	4	3
4	700	3	1
6	800	1	5
Hypophysectomized rats untreated.			
3	200	1	2
3	300	0	3
3	400	0	3
1	600	0	1
Untreated normal controls.			
2	1000	0	2
1	900	1	0

* The treated rats received 1 cc. of a saline emulsion of ox anterior hypophysis. An emulsion of the anterior lobes of 8 glands was made in 80 cc. of physiological salt solution under aseptic conditions. Fresh emulsion was prepared every third day. Injections were administered subcutaneously in amounts of 1 cc. per rat per day for a period of 14 days.

histamine. Rats treated with pituitary emulsion showed none of the usual effects of hypophysectomy.

Further experimentation, however, will be done to determine what fraction of this crude emulsion is responsible for the increase in resistance of hypophysectomized rats. From data previously obtained it is suggested that the adrenotropic fraction in this emulsion may be a protective factor.

The effect of emulsions of anterior hypophysis (ox) on the natural resistance of normal rats to histamine was determined in a second experiment. Twenty-six adult albino rats, 3 to 4 months of age, were divided into 2 groups. Sixteen received daily subcutaneous injections of a saline anterior hypophyseal emulsion in amounts of 1 cc. per rat per day for a period of 2 weeks. Ten were untreated. All were injected with histamine (ergamine acid phosphate) in amounts varying from 900 to 1600 mg. per kg. of body weight. In previous work[3-7] the M.L.D. of this drug in the normal animals of our stock was found to be about 900 to 1,000 mg. per kg. Only an occasional rat sur-

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[3] Marmorston-Gottesman, J., and Gottesman, J., *J. Exp. Med.*, 1928, **47**, 503.

[4] Perla, D., and Marmorston-Gottesman, J., *Am. J. Phys.*, 1929, **89**, 152.

[5] Perla, David, and Marmorston-Gottesman, J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 650.

[6] Marmorston-Gottesman, J., and Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 1022.

[7] Perla, D., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 982.

vived one killing dose of histamine. These rats increased markedly in weight, and at autopsy the suprarenals showed striking hypertrophy as did the gonads, spleen and thyroid. Nevertheless this was not associated with an increase in the natural resistance to histamine. The natural resistance of normal rats was not appreciably raised by repeated injections of a saline emulsion of anterior hypophysis.

TABLE II.
Effect of Repeated Injections of Anterior Hypophyseal Saline Emulsion on the Natural Resistance of Normal Rats to Histamine Poisoning (Ergamine Acid Phosphate).

No. Rats	Amt. Histamine per kg.	Survived	Died
	Normal rats treated with anterior hypophyseal emulsion.*		
6	1100	2	4
6	1200	1	5
3	1400	0	3
1	1600	0	1
	Normal rats untreated.		
2	900	2	0
4	1000	2	2
4	1100	1	3

* The emulsion was prepared as in the previous experiment. Subcutaneous injections were given in amounts of 1 cc. daily for a period of 14 days prior to the administration of histamine.

Summary. The natural resistance of hypophysectomized rats treated with a crude emulsion of fresh anterior hypophysis during a period of 2 weeks following the operation to subsequent injections of histamine is increased almost to the normal level. The natural resistance of normal rats treated with a crude emulsion of fresh anterior hypophysis during a period of 2 weeks to subsequent injections of histamine is not appreciably increased.

8277 P

Effect of Vitamin A on Proliferation of Fibroblasts.

LILLIAN E. BAKER. (Introduced by T. M. Rivers.)

From the Laboratories of the Rockefeller Institute for Medical Research.

The purpose of the experiments reported here was to ascertain whether or not vitamin A is one of the substances needed by fibroblasts for their life and proliferation *in vitro*. Two previous investigators have already attempted to answer this question. Unfortunately, however, they arrived at opposite conclusions. Thus, Burrows[1] compared the change in concentration of vitamin A in chick embryos, as they increased in age, with the change occurring simultaneously in the growth-promoting power of the juice of the embryos. He concluded that vitamin A inhibited growth. Bisceglie,[2] on the other hand, added a trace of the "A factor" to hanging drop cultures of liver and spleen and found it to be growth stimulating. No information was given as to the source of the "A factor", or the extent to which it had been purified.

In the present investigation, crystalline carotene was first tried as a source of vitamin for the cells. No increase in growth was obtained.* Vitamin A was, therefore, prepared from halibut liver oil. Two different concentrates were made. In preparing the first, the oil was saponified, and the vitamin extracted with petroleum ether. The ether was evaporated, and the residue taken up in methyl alcohol. Sterols were then removed by cooling the methyl alcohol solution to 0°C. After this, the vitamin was redissolved in hexane, and more sterols were removed by cooling to —10°C. An oily residue was obtained on evaporation of the hexane. The second preparation was purified to a further extent. In this case, a mixture of CO_2 ice and alcohol was used to freeze out the sterols from the methyl alcohol solution. The residue from the methyl alcohol was then dissolved in pentane and the freezing with CO_2 ice repeated. After this, the vitamin was dissolved in petroleum ether, adsorbed on alumina, and eluted with chloroform.[3] A product of a high degree of purity was thus obtained. For the purpose in hand, it did not seem advisable to attempt any further purification, at least, not until such a time as the vitamin will have been obtained in crystalline form.

In order to bring the vitamin into a form in which it could be utilized by the cells, an attempt was made to dissolve it in serum. Samples of chicken sera were allowed to stand overnight at room temperature with small quantities of the vitamin preparations. When tested by the antimony trichloride reaction,[4] these sera were found to have increased their original vitamin A content approximately 200 times. A second portion of each sample of serum (for use in the control cultures) was likewise kept overnight at room temperature without being exposed to the vitamin.

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[1] Burrows, M. T., *Am. J. Physiol.*, 1926, **77**, 24.

[2] Bisceglie, V., *Arch. Entwiklngsmechn. Organ.*, 1926, **108**, 708.

* Further work is being done to ascertain the reason for the different results obtained with carotene and vitamin A.

[3] These methods of preparation were taken largely from the work of Holmes and his co-workers. Holmes, H. N., Cassidy, H., Hartzler, E., and Manly, R., *Science*, 1934, **79**, 255; Holmes, H. N., Lava, V. G., Delfs, E., and Cassidy, H., *J. Biol. Chem.*, 1932, **99**, 417.

[4] Morris, E. R., and Church, A. E., *J. Biol. Chem.*, 1929, **85**, 477.

The "vitamin sera" were then tested for their effect on the growth of heart fibroblasts. Two different strains of fibroblasts were used: one, the 23-year-old strain of Carrel; the other, a new strain that had been derived quite recently from the heart of an embryonic chick. Each culture was divided into 2 equal parts. These were embedded in separate flasks in coagula made of horse plasma.† One of these cultures received the vitamin serum; the other, an equal amount of the control serum. All of them were given a nutrient fluid that contained Witte's peptone, cysteine, hemin, insulin, thyroxine, glucose,[5] the salts of Tyrode solution, and some normal serum.‡ The vitamin and control sera were used in amounts ranging from 0.002 to 0.05 cc. per cc. of medium. The normal serum was given in such amounts as to make the total concentration of serum in the medium equal to 10%.

When used at the highest concentrations, the "vitamin sera" were toxic. At lower concentrations, however, they had a most beneficial effect on the cells. The cells that received the vitamin proliferated much more rapidly than did the control cells. In many instances, they formed colonies 3 times as large as those in the controls. The largest growth was generally obtained when the "vitamin sera" were used as 1% of the medium. In addition to promoting growth, the incorporation of vitamin in the medium prevented the accumulation of large amounts of fat in the cells, a reaction that was quite characteristic of the control cultures. It also enabled the cells to live and proliferate in the artificial medium for a much longer time than they could in the absence of the vitamin. As far as could be observed, the 2 preparations of the vitamin gave identical results, in spite of the differences in the extent to which they had been purified.

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† The horse plasma used in these experiments was supplied in an aseptic condition by the New York City Department of Health. The author wishes to express her appreciation of this favor.

[5] The constituents used by Vogelaar and Erlichman in their feeding solution. Vogelaar, J. P. M., and Erlichman, E., *Am. J. Cancer*, 1933, **18**, 28.

‡ When horse plasma is used for making the coagulum, it is necessary to add some chicken serum to the medium to obtain growth.

8278 P

Influence of Certain Dye-Stuffs on Fermentation and Respiration of Yeast Extract.

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An aqueous extract of yeast can be prepared which is free of cells but which will actively ferment glucose to alcohol and CO_2. Such an extract consumes only a small amount of oxygen and this small amount exerts no measurable effect on the rate of fermentation. Consequently the extract exhibits no Pasteur reaction. If, however, a dyestuff, which is reversible with respect to oxidation and reduction and which can be reduced by the extract and oxidized by oxygen, is added to the extract, an appreciable oxygen consumption ensues. According to Lipmann[1] this oxygen consumption may or may not inhibit fermentation. He concludes that the deciding factor is the oxidation-reduction potential which the dyestuff imposes on the fermenting mixture. If the potential range of the dye added is relatively negative no inhibition occurs. If it is relatively more positive and oxygen is supplied at a rate sufficient to keep a part of the dye in the oxidized form, inhibition does occur and, according to his results, may be reversed if the potential is lowered by making the experiment anaerobic and adding a reductant (ascorbic acid) plus diphosphate. He regards this as an example of the Pasteur reaction and considers the mechanism of this reaction to be the reversible oxidation of some component of the fermentation system which is active only in the reduced form. If this explanation of the Pasteur reaction is true, and it seems to have found a ready acceptance by some investigators,[2, 3] it would have a most important bearing on the fundamental aspects of respiration and fermentation. It seemed to us desirable to try and check this general idea.

The yeast used was a bottom yeast kindly supplied to us by Ruppert's brewery. It was pressed at 800 lb. pressure, dried in the air at 25°C., ground to a fine powder and stored in the ice box. The extract was prepared from a portion of this powder with 3 volumes of distilled water according to Lebedev.[4] Various samples had a pH of 6.10, varying no more than ±0.05 unit, measured with the glass electrode; and were slightly less than 0.1 m. in inorganic phosphate. All the extracts prepared exhibited strong reducing properties. Anaerobically methylene blue and indigo disulfonate were rapidly and completely reduced. Even rosindulin G G was completely, though very slowly reduced. As judged by the iodine titration, oxidized glutathione was reduced at an appreciable rate. Benzyl viologen was not reduced.

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[1] Lipmann, F., *Biochem. Z.*, 1934, **268**, 205.

[2] Kluyver, A. J., and Hoogerheide, J. C., *Biochem. Z.*, 1934, **273**, 197.

[3] Morgulis, S., and Munsell, J. D., *Biochem. Z.*, 1935, **278**, 89.

[4] Lebedev, A. v., *Z. Physiol. Chem.*, 1911, **73**, 447.

The general effect of small amounts of dyes on fermentation is to shorten the induction period and accelerate the beginning stages. Under anaerobic conditions this is the only effect of small amounts of dye, but larger amounts may distinctly inhibit although there may be an initial period of acceleration. Under aerobic conditions oxygen is consumed and fermentation is usually inhibited after an initial period of marked acceleration, but the rate at which the inhibition sets in varies considerably from dye to dye. Thus in agreement with Lipmann we have found that 1 naphthol 2 sulfonate indophenol inhibits much more strongly than thionine. The concentration range tested was from 7×10^{-4} m. to 4.9×10^{-3} m. We have also found that pyocyanin inhibits about as strongly as the indophenol. Indigo disulfonate also exerts an appreciable inhibition, while phenosafranine does not inhibit although it brings about a considerable oxygen consumption.

A consideration of these results together with the potential range of the above dyes indicates that with our material the potential range is not the factor that determines the inhibition of fermentation. A comparison of the oxygen consumed by the different dyes and at different temperatures (experiments were done at 37°, 28°, and 24°C.) shows that there is no correlation between the amount of oxygen consumed and the degree of inhibition. The inhibition obtained with all the dyes except naphthol sulfonate indophenol is always irreversible and is due to the destruction of enzymes. Thus the carboxylase activity of the extracts is completely destroyed. Under the proper conditions a partially purified preparation of carboxylase[5] will restore fermentation. The carboxylase activity of this purified preparation is readily destroyed by treatment with dye plus oxygen. Carboxylase is, however, not the only enzyme inactivated. Phosphorylation and phosphate hydrolysis also cease. The enzymes responsible for the reducing action of the yeast remain active after fermentation has ceased, but they are also gradually inactivated. Naphthol sulfonate indophenol also brings about this irreversible inhibition, particularly at 37°, but in addition it can inhibit fermentation in such a way that making the experiment anaerobic and adding ascorbic acid and hexose-diphosphate as Lipmann reports restores the fermentation. This inhibition is, according to our experiments, due to the removal of the diphosphate or the normal intermediates that result from it and the restoration is due to the addition of more diphosphate and not to the reduction of the dye. The addition of sufficient diphosphate at the beginning diminishes this inhibition. The reduction of the dye after it has inhibited fermentation does not restore the process, but the addition of diphosphate without reducing the dye does restore it.

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[5] Axmacher, Fr., and Bergstermann, H., *Biochem. Z.*, 1934, **273**, 259.

8279 C

Experiments on Purification of Bacteriophage, and a Respiratory Pigment in Escherichia Coli Communis.

KARL MEYER, RICHARD THOMPSON, DEVORAH KHORAZO, AND JOHN W. PALMER.

From the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, and the Institute of Ophthalmology of the Presbyterian Hospital, New York City.

During a study of the bacteriolytic enzyme, lysozyme,[1] we wished to compare its properties with those of bacteriophage. The method used for the purification of lysozyme was not applicable to bacteriophage because of the extreme sensitivity of phage to the common organic solvents.

Previous efforts at purification of bacteriophage have usually employed physico-chemical methods: ultrafiltration through membranes of graded porosity,[2] adsorption,[3] and more lately centrifugation.[4] In the present work the removal of undesired culture medium and bacterial components was attempted by chemical methods and the behavior of phage toward some chemical agents was observed.

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[1] Meyer, K., Thompson, R., Palmer, J. W., and Khorazo, D., *Science*, 1934, **79**, 61; complete report in press.

[2] Elford, W. J., and Andrews, C. H., *Brit. J. Exp. Path.*, 1932, **13**, 446.

[3] Gildemeister, E., and Herzberg, K., *Zentralbl. Bakt.*, 1924, **91**, 228; **93**, 402; Kligler, I. J., and Olitzki, L., *Brit. J. Exp. Path.*, 1931, **12**, 172; Clifton, C. E., Proc. Soc. Exp. Biol. and Med., 1930, **28**, 32.

[4] Schlesinger, M., *Z. Hygiene*, 1932, **114**, 161.

The phage and coli strains here used were obtained from Dr. M. Holden, of the Department of Bacteriology. The organism gives the sugar reactions of *Escherichia coli Communis.* The phage was isolated from a normal stool several years ago and has been propagated since on the coli strain.

In obtaining phage for concentration an 18-hour culture was inoculated into a liter of beef heart infusion broth (pH 7.4-7.6) and incubated at 37° until clouding was plainly visible. A small amount of the phage broth was added and the flask was incubated another 18 hours. Complete clearing usually occurred and the broth was filtered through a Berkefeld N filter.

In titrating the various phage preparations 0.5 cc. of progressive dilutions were placed in sterile Wassermann tubes and 4.5 cc. portions of a 3 to 4-hour culture of the organism were added. A control tube of 0.5 cc. of sterile broth with the culture was used with each series. After 18 hours at 37° readings were made, the highest dilution giving complete clearing being designated as the end point.

In preparing the concentrates of phage, 500-1000 cc. of phage broth were made slightly alkaline (just pink to phenolphthalein paper) and commercial colloidal iron (60-65 cc. per liter of broth) was slowly added with vigorous stirring. The bulky precipitate, which carried down only a small amount of phage, was removed. A 4% aqueous solution of naphthol yellow S (sodium flavianate) was added, and the solution was brought to a pH of about 4 with HCl. A negligible amount of phage was carried down, except in a few unexplained instances, where it was apparently adsorbed on some polypeptide since it could be washed out with acidified water. The acid solution was treated with solid $BaCl_2$ and neutralized in steps with NaOH until a pH of about 7.6 was reached. The resulting precipitates were centrifuged off and discarded. The pH was then brought from about 7.6 to 8.4. This last precipitate contained the phage in very good yield. After the precipitate was washed several times with water (whereby no phage passed into the washings), it was suspended in about 50 cc. of water acidified with a small amount of H_2SO_4 and the rest of the barium removed with Na_2SO_4. The mixture was left overnight in the cold. The supernatant fluid after centrifugation contained the phage. The solutions at this stage had a slight yellow color from the flavianic acid used.

The phage was inactivated by precipitation with alcohol and to a lesser degree with acetone. Evaporation *in vacuo* over P_2O_5 while the material was in a frozen state destroyed most of the activity of the phage. In a beam of strong light (from a slit lamp) there was only a faint Tyndall effect. One preparation contained only 5.5% nitrogen on an ash-free basis, and was active in a dilution greater than $1:10^9$ (end point not reached). In a 1% solution the xanthoproteic and Greenberg phenol reactions were positive, the Molisch weakly positive, and biuret, Millon, glyoxylic acid, nitroprusside, and alkaline lead reactions were negative.

The following example indicates that the precipitation of the phage by barium is not due to adsorption, a fact also suggested by the failure of colloidal iron to precipitate phage at the same pH. Phage broth was carried through the procedure outlined above as far as the barium precipitation. The final barium precipitate was centrifuged off, washed with alkaline water, suspended in water, and made neutral. The supernatant fluid was freed of barium with Na_2SO_4, and found to be active in $1:10^2$ dilution. The remaining barium precipitate was suspended in water, acidified with H_2SO_4, and barium was removed with Na_2SO_4. The solution (50 cc.), active to $1:10^7$, contained per cc. 0.628 mg. organic matter with 9.17% nitrogen. Obviously the bulk of the phage was not removed from the barium precipitate until the suspending medium was acid enough to liberate weak acids from their barium salts.

Another similar preparation was active to $1:10^6$ (end point not reached). After dialyzing in a collodion membrane against distilled water it was active to $1:10^5$. After evaporation of 15 cc. while frozen, 10 mg. of a very soluble material of slightly yellow color was obtained, which when dissolved in 5 cc. of water was active to $1:10^2$.

The nature of bacteriophage is still unknown. The precipitate described, probably still quite impure, indicates that the phage is of an acidic nature, forming an insoluble barium salt.

A similar procedure was used by Hosoya, Nagase, and Yoshizumi,[5] for the purification of toxins and of phage. They found phage completely precipitated by zinc hydroxide. The zinc precipitate was redissolved in acid, the solution dialyzed and again precipitated by zinc acetate and ammonia. The lysin did not dialyze and gave negative biuret, Millon, xanthoproteic, diazo, Molisch, and ninhydrin reactions.

Recently Schüler,[6] working with phage prepared by Schlesinger,[4] failed to find any metabolism and concluded it was not a living organism. He found a phosphatase associated with the phage, but could not show that the phage and phosphatase were identical. Our phage concentrates also failed to show either oxygen uptake or glycolysis.

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[5] Hosoya, S., Nagase, K., and Yoshizumi, T., *Jap. J. Exp. Med.*, 1932, **10**, 101.

[6] Schüler, H., *Biochem. Z.*, 1935, **276**, 254.

The results of our observations are also hardly compatible with the theory of a living organism. It seems probable that further experiments on the particle size of a purified preparation of phage should yield helpful information.

Note on a Respiratory Pigment in Escherichia coli Communis.—During the work with acetone-dried coli a pigment was observed on fractionation. The dried bacteria were extracted with 200 cc. of N/200 NaOH per gram. After centrifuging, the colorless supernatant liquid was acidified with HCl, the precipitate removed, and the solution precipitated with flavianic acid. To the supernatant solution 4% phosphotungstic acid was added. After standing cold over night the flesh colored precipitate was centrifuged off, washed, and dissolved in a small amount of alkaline water, giving a colorless solution. This solution was brought to 2N H_2SO_4 (the color was restored upon acidification) and extracted with a mixture of equal parts of amyl alcohol and ether; the red organic solution was washed 5 times with water until the washings no longer had a yellow color (flavianic and phosphotungstic acids being removed) and then evaporated *in vacuo*. The residue was free from phosphotungstic acid. It had a violet-red color, was insoluble in acid and alkaline water (in contrast to the substance before treatment with phosphotungstic acid), chloroform, petroleum ether, and acidified ether, and was soluble in amyl alcohol and pyridine.

The pyridine solution had an orange-red color and a greenish-brown fluorescence. The color of the solution disappeared on addition of bisulfite and was restored by peroxide. In a grating spectroscope the pyridine solution showed 2 absorption bands which were not very sharp. The larger was at 543 mμ, and the smaller at 497 mμ. For comparison, a pyridine solution of prodigiosin, which showed no fluorescence, had a larger absorption band at 532.5 mμ, with a smaller at 494 mμ.*

This pigment, while somewhat resembling prodigiosin, is distinctly different from it. It does not seem likely that this pigment is derived from cytochrome since its reduced form is colorless. Apparently in the bacteria it is bound on protein and present in the reduced state.

The pigment seems to be characteristic of this organism, since its preparation was repeated a number of times with the same results and by the same procedure no such pigment could be obtained from sarcinae or from *B. coli Communior.*

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* We thank Dr. C. B. Coulter of the Institute of Public Health for the spectroscopic observations.

8280 P

Agglutination and Precipitation Between Hemolytic Streptococci of Various Groups and Sera of Rheumatoid Arthritis Patients.

CURRIER McEWEN, HERBERT CHASIS, AND R. C. ALEXANDER. (Introduced by E. P. Ralli.)

From the Department of Medicine, New York University College of Medicine, and the Third (New York University) Medical Division, Bellevue Hospital, New York City.

It has been established[1, 2, 3] that hemolytic streptococci are agglutinated by sera of patients with rheumatoid arthritis. It has also been shown[4, 5] that the C substance of hemolytic streptococci[6] gives precipitation with sera of patients with this and other diseases. Since only strains of hemolytic streptococci isolated from human sources had been used in the investigations quoted, the present study was undertaken to observe the results obtained when antigens for agglutination and precipitation were prepared from hemolytic streptococci of Lancefield's groups A, B, C, D, E, F, and G.[7] Strains of groups B through G were kindly provided by Dr. R. C. Lancefield.

Agglutination tests were done with strains of groups A through E only. Eighteen-hour living broth cultures in 0.5 cc. quantities were mixed with 0.5 cc. of the various dilutions of serum obtained from patients with rheumatoid arthritis. Tests were read after 2 hours at 56°C. and 20 hours in the ice box. The results (Table I) indicate that, while the strongest agglutination usually occurred with strains of Group A, definite reactions were obtained also with those of other groups.

It seemed probable that the cross reactions were due to non-group-specific fractions common to all the strains, and to test this the work

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[1] Nicholls, E. E., and Stainsby, W. J., *J. Clin. Invest.*, 1931, **10**, 323.

[2] Dawson, M. H., Olmstead, M., and Boots, R. H., *J. Immunol.*, 1932, **23**, 187.

[3] Keefer, C. S., Myers, W. K., and Appel, T. W., *J. Clin. Invest.*, 1933, **12**, 267.

[4] Seegal, D., Heidelberger, M., Jost, E. L., and Lyttle, J. D., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 582.

[5] Dawson, M. H., Olmstead, M., and Jost, E. L., *J. Immunol.*, 1934, **27**, 355.

[6] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 481.

[7] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

TABLE I.

Agglutination of Hemolytic Streptococci of Groups A, B, C, D, and E (Lancefield) by Sera of Patients with Rheumatoid Arthritis.

			Sera of Patients with:					
			Rheumatic Fever*	Rheumatoid Arthritis				
Group.	Strain	Antigen Control	Wig.	Gold.	Lev.	Car.	Sil.	John.
Group A	NY5	—	—	640+	640+	640+	640+	640+
	McEv	—	—	640	160	80	320	
	Tl	—	40	160	640	640	320	
	Foley	±	20	320	640	—	640+	
	A194	—	—	640+	320	640	640+	
	A216	—	40	320	160	320	320	
	A59	—	—	320	320	160	320	
	N107	—	—	80	80	160	320	
	N71	+	—	160	80	640+	640	
	N96	—	—	640+	640	640+	640+	
	A25A	—	—	160	320	640	640+	
	N110	—	—	20	80	80	640+	
	N56	+	+	320	160	640	640	
	N72	—	160	320	640	320	640+	
	A253	±	—	640+	640+	160	640+	640
	Sylv	++	Spontaneous Agglutination					
	A26B	++	"		"			
	N15	++	"		"			
Group B	K107	—	—	—		20	80	
	K126	—	—	—		20	160	
	K198	—	—	—			80	80
	V8	±	—	—			40	20
	B116	±	20	80			80	80
	K127	—	20	—			40	—
Group C	P230	—	—	—	80	160	640+	
	K155N	±	—	40	40	40	640	
	K155C	+	—	—	20	20	640	
	K155L	—	—	—		80	640	
	K155K	±	—	20		—	320	
	K155M	—	—	—			80	40
	K132	—	—	—			40	20
	K150A	±	—	—			320	80
Group D	C2	±	—	—			—	—
	C5	—	20	20			40	40
	C6	—	20	—			40	20
	C3	—	—	—			—	—
	C1	+	—	—			—	—
Group E	K131	—	—	—			—	—
	K129	±	40	20			—	—
	K128	+	—				40	—

* Serum of patient with rheumatic fever included as a control.

Figures indicate dilutions through which each strain was agglutinated by the respective sera.

640+ signifies that agglutination at this titer was sufficiently strong to indicate that it would have been positive in higher dilutions had they been tried.

— = negative. Blank = not done.

was repeated using the precipitin reaction against the group-specific C substance. Crude C extracts of strains of groups A through G were prepared by Lancefield's method.[7] These were tested against sera of 11 rabbits immunized with representative strains of each group. In each instance antigen dilutions were prepared by adding 0.4, 0.1, 0.025, and 0.01 cc. of extract to sufficient physiologic saline to make a final amount of 0.4 cc. Following the addition of 0.2 cc. of serum, the tubes were kept 2 hours at 37°C. and 20 hours in the ice box, and were then read before and after centrifugation. As found by Lancefield,[7] the various groups were specific and showed no significant cross reactions except in the case of serum from one rabbit immunized with a group E strain, which gave distinct crossing with extracts of every group. This apparently was a peculiarity of the animal, for other rabbits immunized with the same strain reacted in the usual manner. When sera of patients with rheumatoid arthritis, rheumatic fever, and convalescent scarlet fever were substituted for those of immune rabbits, however, definite crossing was encountered, occasionally with extracts of all groups and frequently with those of groups A, B, and G. These precipitates were distinct only after centrifugation.

There are at least 3 possible explanations of the unexpected cross reactions: (1) that they are not of true antigen-antibody nature, (2) that they represent responses of the anamnestic type in patients who previously harbored hemolytic streptococci of different groups, or (3) that they are due to the presence of multiple antigens in the crude extracts and to varying capacity of different individuals to form multiple antibodies following infection. The latter seems most probable, especially in view of the wide crossing encountered with one of the immunized rabbits. Work is in progress to test the validity of these possibilities.

Until the mechanism underlying these cross reactions is understood it is believed that they must be taken into account in attributing etiologic significance in rheumatoid arthritis to agglutination of hemolytic streptococci or to precipitation with crude C extracts.

8281 P

Acetyl Choline and the Blood Sugar Level.

M. CAROLINE HRUBETZ. (Introduced by H. B. Williams.)

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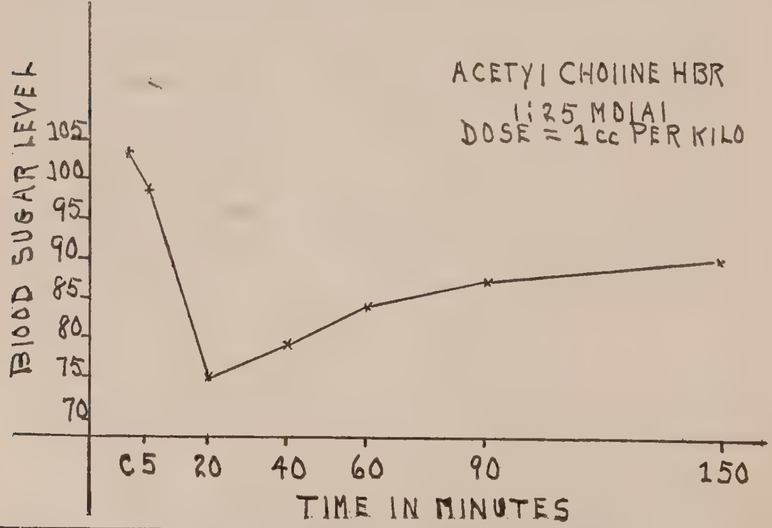
Hunt,[1] working with the blood pressure reducing principle of suprarenal and brain, ascribed the fall in blood pressure to acetyl choline. He stated that he was "inclined to think that it is due to an effect upon the terminations of the vagus in the heart." Dale[2] reported "it can now be definitely stated that the vagus impulses produce their effects by liberating acetyl choline among the fibers of the muscular wall of the heart." In another paper, the author[3] reports an attempt made to determine whether or not there might be a parasympathetic innervation to the Islets of Langerhans. From the results obtained it appears that if the parasympathetics be motor to the islets, pilocarpine, in this case, does not act by stimulation since this drug causes a rise in the blood sugar level. There is the possibility, however, that the parasympathetics are inhibitory. Since it has been quite satisfactorily demonstrated that this system acts by liberating acetyl choline, it was thought worth while to try the effects of this drug upon the blood sugar level.



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[1] Hunt, Reid, and Takeau, *Brit. Med. J.*, 1906, **11**, 1788.

[2] Dale, H. H., *Science*, 1934, **80**, 1.

[3] Hrubetz, M. C. In press.

Acetyl choline Hydrobromide was used in concentration of 1:25 molal. The dosage used was 1 cc. per kilo. All experiments were performed upon normal-fed rats. The 0.2 cc. Somogyi[4] modification of the Shaffer-Hartmann blood sugar method was used. Approximately 50 observations were made upon each point of the curve with the same number in the control series. As can be seen from the accompanying chart, there is a sharp fall in the blood sugar level, the greatest change occurring at the 20-minute interval. The blood sugar has not returned to the original level in 2½ hours.

From these results it appears that the vagus may carry motor impulses to the Islets of Langerhans—if this fall in blood sugar is produced by a liberation of insulin. However, there may be some other mechanism involved, such as the inhibition of glycogenolysis. At the present time, we are unable to explain the mechanism involved in this fall in the blood sugar level. Further work is being done on the subject.

8282 C

Further Observations on the Poliocidal Property of Pregnant Mare Serum.*

CLAUS W. JUNGEBLUT.

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

We have previously been interested in the changes that occur with pregnancy in man and animals with reference to the ability of the serum and of other biological substances to neutralize the virus of poliomyelitis *in vitro*.[1, 2] In an earlier paper[2] it was reported that the serum of 2 pregnant mares when combined in high dilutions with this virus was capable of bringing about its inactivation. No data were available at that time to indicate whether the normal serum of the same non-pregnant animal possessed similar properties. Again, since our work included tests on 2 animals only, it was unknown how regularly the same phenomenon could be elicited with the serum of other pregnant mares. The most important gap in the observed

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[4] Somogyi, M., *J. Biol. Chem.*, 1926, **70**, 599.

* Under a grant from the Rockefeller Foundation.

[1] Jungeblut, C. W., and Engle, E. T., *J. Immunol.*, 1933, **24**, 267.

[2] Jungeblut, C. W., Meyer, K., and Engle, E. T., *J. Immunol.*, 1934, **27**, 43.

TABLE I.

Virucidal Tests and Gonadotropic Hormone Determinations with Pregnant and Normal Mare Sera.

Mare No.	Type of serum Pregnancy	Type of serum Days Pregnant	Date bled	Hormone test	Virucidal Test 0.8 cc. of serum + 0.2 cc. 10% virus emulsion: Test Monkey No.	Dilution of serum	Result			Control Monkey No.	Complete paralysis days
15	I	72	May, '33* †	Not done	J20	Undiluted	No	paralysis		J11	6
					M13	1:5	”	”		M32	8
					J24	1:10	”	”		J11	6
					J60	1:20	”	”		J11	6
					J70	1:40	”	”		J69	9
					J66	1:60	Complete	”	9 days	J69	9
	Normal	61 days after pregnancy	Mar., '34 †	” ”	L69	Undil.	Partial	”	12 d.	L67	10
15	II	48	June, '34	.25 cc. 3+ .1 3+ .05 2+ } 3+	M77	”	No	”		M76	7
					M72	1:10	”	”		M76	7
					M86	1:20	”	”		M79	8
					M87	1:40	”	”		M79	8
					N15	1:60	Partial	”	9 d.	N25	11
	II	132	Sept. '34	.25 2+ .1 1+ .05 — } 2+	N7	Undil.	No	”		N3	9
					N38	1:20	”	”		N25	11
					N18	1:40	”	”		N25	11
					N70	1:60	Partial	”	10 d.	N53	7
	II	198	Nov., 34	.25 1+ .1 ± .05 — } 1+	N44	Undil.	Complete	”	10 d.	N26	7
					N68	”	”	”	9 d.	N53	7
	Normal	9 days after pregnancy	Apr., '35	.25 — .1 — .05 — } 0	Q17	”	No	”		Q13	8
	Normal	56 days after pregnancy	June, '35	Not done	Q59	”	”	”		Q84	9
					Q89	1:5	Complete	”	9 d.	Q90	9
22	I	96	June, '33 *	” ”	J71	1:20	No	”		J69	9
					J72	1:40	”	”		J69	9
					J73	1:60	Complete	”	11 d.	J69	9

	Normal	4 days after pregnancy	Mar., '34	"	"		L68	Undil.	"	"	9 d.	L67	10
22	II	57	Sept., '34	.25 cc. .1 .05	4+ 4+ 4+	4+	N17 N46	" 1:5	No Complete	" "	11 d.	N25 N26	11 7
	II	140 (horse lost fetus)	Dec., '34	.25 .1 .05	— — —	0	N64	Undil.	"	"	6 d.	N53	7
27	I	71	June, '34	.25 .1 .05	2+ — —	1+	M73 M74	" 1:10	" "	" "	8 d. 11 d.	M76 M76	7 7
	I	155	Sept., 34	.25 .1 .05	1+ — —	1+	N8 N19 N20	Undil. " 1:5	Partial Complete "	" " "	14 d. 17 d. 11 d.	N39 N25 N25	9 11 11
	I	226	Dec., '34	.25 .1 .05	± — —	0	N445 N59	Undil. 1:5	No Complete	" "	8 d.	N26 N53	7 7
	Normal	29 days after pregnancy	Apr., '35	.25 .1 .05	— — —	0	Q16	Undil.	"	"	8 d.	Q13	8
20	I	64	Aug. '34	.25 .1 .05	4+ 4+ 4+	4+	N10 N47	" "	" Partial	" "	7 d. 6 d.	N3 N26	9 7
	I	183	Dec., '34	.25 .1 .05	— — —	0	N63	"	Complete	"	7 d.	N53	7
8	I	65	Sept., '34	.25 .1 .05	— — —	0	N9	"	"	"	8 d.	N3	9
	I	293	Apr., '35	.25 .1 .05	± — —	0	Q15	"	"	"	8 d.	Q13	8

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* These data have been previously reported in the *J. Immun.*, 1934, **27**, 43.

† These 2 sera have been kindly examined in parallel tests by Dr. N. P. Hudson of the University of Chicago. In agreement with our own results he reported the serum of the pregnant animal as neutralizing and the normal serum as non-neutralizing.

facts, perhaps, was the absence of information as to whether or not the poliocidal power ran parallel with the content of the serum of gonadotropic hormone. It is the purpose of this communication to supply the experimental facts which will answer the questions raised above.

Serum obtained from 5 mares at various stages of pregnancy and, in most cases, after foaling also, were available through the courtesy of Dr. Fred F. McKenzie of the Agricultural College of the University of Missouri, Columbia, Mo. We should like to express our sincere gratitude to Dr. McKenzie for supplying us with this material, for without his untiring cooperation these studies would have been impossible. The samples represented unpreserved, fresh bleedings which were shipped by express. Immediately upon arrival at this laboratory they were tested for virucidal property and for hormone content. The technique of the virucidal test followed the same general principles previously employed in work of this kind, *i. e.,* 0.8 cc. of serum or serum dilution were combined with 0.2 cc. of a 10% virus suspension; the mixtures were incubated for 1½ hours at 37°C., kept in the icebox over night, and the following morning injected intracerebrally into individual monkeys. The animals were carefully observed for one month and absence or presence of paralysis was noted. Control monkeys receiving the same dose of virus mixed with saline accompanied each test. The test for gonadotropic hormone was carried out by injecting duplicate female immature mice with 3 doses of the serum, *i. e.,* 0.25 cc., 0.1 cc., and 0.05 cc., daily for a period of 3 days and examining the animals on the fourth day for evidence of uterine and ovarian stimulation. In reading these tests we were kindly assisted by Dr. E. T. Engle and Miss Parsons.

A total of 19 samples were tested for poliocidal properties, mostly in quantitative titrations, and an almost equal number of parallel hormone determinations were carried out. The data are brought together in Table I.

In analyzing the contents of this table certain conclusions suggest themselves. First, it appears that poliocidal substances are not present in the serum of all mares during pregnancy at the intervals tested in our experiments. Thus, of 5 pregnant mares such substances could be demonstrated, at some time or another, in only 3 animals, 2 failing to exhibit this property on repeated tests. Second, there is no evidence of any clear-cut relation between the poliocidal property of the serum and its content of gonadotropic hormone. While it is true that in some cases poliocidal sera also showed a high hormone concentration and sera with low or no hormone content possessed no poliocidal properties, in other instances sera which gave a high hormone titer failed to neutralize the virus. Third, our data although spreading over several months of pregnancy are insufficient to indicate clearly whether the concentration of poliocidal substances is highest at any given point of gestation. From the data on hand it would seem that there is considerable individual variation between different horses. Fourth, the experimental evidence shows unmistakably that the power of the serum to inactivate the virus is dependent upon changes that occur with pregnancy. Thus, in 2 cases when the normal serum was completely devoid of poliocidal principles, such substances appeared in small amounts coincidental with pregnancy. In a third case, poliocidal substances were not found in the normal serum taken before pregnancy but were apparently present in appreciable amounts in serum drawn after termination of pregnancy. In this latter instance, titrations of the serum during pregnancy indicated exceptionally high concentrations. Of particular interest in this connection are the 2 horses, No. 15 and No. 22, both of which were observed during 2 successive pregnancies. While mare No. 15 went twice through a normal course of pregnancy yielding sera which reacted completely alike, mare No. 22 lost its foal during the latter course of the second pregnancy. With resorption of the fetus both the poliocidal property and the hormone titer disappeared simultaneously.

In summarizing, we should like to emphasize the following points: The presence of poliocidal substances in the serum of pregnant mares can be definitely traced to the immediate effects of pregnancy. This property is present in some and absent in other pregnant mares. It seems to vary with individual horses irrespective of the gonadotropic hormone titer of the serum. When present during pregnancy, this property bears no clear relation to the concentration of gonadotropic hormone in the serum. Further work will perhaps show whether the accumulation of poliocidal substances can be linked more definitely with some other constituent of the serum which increases with pregnancy.

8283 C

Blood Amylase Response to Acetyl-Beta-Methylcholine Chloride in Pancreatectomized Dogs.[*]

LESTER TUCHMAN, ARTHUR SCHIFRIN, AND WILLIAM ANTOPOL.
(Introduced by George Baehr.)

From the Laboratories of the Mount Sinai Hospital, New York City, and the Bayonne Hospital and Dispensary, Bayonne, New Jersey.

It has been shown that there is a conspicuous rise of blood amylase after the administration of acetyl-beta-methylcholine chloride.[†][1] In order to determine the possible pancreatic origin of this increase, the experiments were repeated both before and after pancreatectomy. Determinations of the blood amylase in 5 dogs showed constant rises in the blood amylase titer after the intramuscular administration of the choline ester. The same dogs were then pancreatectomized under pernoston anesthesia and the extent of the blood amylase response to acetyl-beta-methylcholine chloride was again estimated. The viscosimetric method utilized for these determinations was identical with that reported in the original communication.[1] Determinations were made on the insulin-treated pancreatectomized animal in the fasting state, without the administration of insulin on the day of the experiment. A fasting specimen of blood, and one collected 4 hours after the intramuscular administration of acetyl-beta-methylcholine chloride was deemed sufficient for this investigation. Repeated post-pancreatectomy estimations revealed no blood amylase increase (Table I), the rise obtained in the unoperated animal being totally or almost totally abolished after removal of the pancreas.

In one dog, it was noted that the blood amylase could again be elicited about 4 weeks after pancreatectomy. We suspected, therefore, that remnants of the pancreas remained which later regenerated and hypertrophied. With this in mind, an exploratory laporatomy was performed, and 8.5 gm. of pancreatic tissue was found and removed. After this secondary operation, no amylase response to acetyl-beta-methylcholine chloride could be elicited after a period of 55 days. Table II.)

As additional controls, determinations were made on 4 dogs after

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[*] Aided by a grant from the Committee on Scientific Research of the American Medical Association.

[†] Mecholyl-Merck.

[1] Antopol, W., Schifrin, A., and Tuchman, L., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 383.

TABLE I.

Date	Fasting blood amylase units	Blood amylase units 4 hr. after injection of 10 mg. acetyl-beta-methylcholine chloride	% increase
DOG 467.			
2/15/35	12	75	525
2/16/35	Pancreatectomy		
2/21/35	6	10	66
3/13/35	5	5	0
3/27/35	5	5	0
3/29/35	Died probably in acidosis. Post mortem showed no significant anatomical findings. No pancreatic tissue found.		
DOG FRITZY			
12/28/34	33	75	127
12/29/34	Pancreatectomy		
1/2/35	21	21	0
1/9/35	11	13	13
1/10/35	Died. Post mortem showed severe broncho pneumonia. No pancreatic tissue found.		
DOG 20.			
1/10/35	20	300	1400
1/14/35	25	270	940
1/17/35	Pancreatectomy		
1/21/35	12	12	0
1/21/35	Died. Post mortem showed severe broncho pneumonia. No pancreatic tissue found.		
DOG 436.			
2/13/35	17	67	294
2/21/35	19	50	163
2/26/35	Pancreatectomy		
2/28/35	60	60	0
3/4/35	19	26	37
3/13/35	14	13	0
3/20/35	13	12	0
3/27/35	18	18	0
4/12/35	13	13	0
4/25/35	20	17	0
4/30/35	13	14	0
5/16/35	13	14	0
6/20/35	13	13	0
	Dog still alive		

pernoston anesthesia alone, and after abdominal operations. Although the blood amylase in some of these failed to show an appreciable rise after the procedure, the rise could again be elicited after a period of from one to 2 days.

Summary. The blood amylase response to acetyl-beta-methylcholine chloride cannot be elicited in dogs after total pancreatectomy.

TABLE II.

Date	Fasting blood amylase units	Blood amylase units 4 hr. after injection of 10 mg. acetyl-beta-methylcholine chloride	% increase
	DOG 16.		
11/28/34	18	75	317
11/29/34	21	85	310
12/1/34	Pancreatectomy		
12/4/34	34	35	3
12/8/34	15	18	20
12/17/34	13	17	22
12/18/34	11	13	18
12/28/34	37	43	16
1/2/35	15	40	166
1/9/35	13	30	131
1/22/35	15	31.5	110
2/19/35	17.5	37.5	115
3/8/35	Secondary Removal of Portion of Pancreas		
3/13/35	11	10	0
3/20/35	9	9	0
4/1/35	10	10	0
4/2/35	11	11	0
4/24/35	14	14	0
	Dog killed—no pancreatic tissue found.		

8284 C

An Attempt to Demonstrate Local Formation or Concentration of Virucidal Antibodies.*

MARGARET HOLDEN AND PAUL S. STRONG. (Introduced by F. P. Gay.)

From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University.

By the local injection of bacteria it is sometimes possible to show an antibody concentration at the site of inoculation greater than that in the serum. Cannon[1] found that the extracted juice of inoculated skin exceeded the antibody titre of the serum, as well as that of uninoculated skin. He suggests that local formation of antibody in the injected area is in part responsible for the effect. Seegal and Seegal[2] noted that there was a concentration of specific agglutinins in the fluid of the tissues surrounding the anterior chamber of the eye following local injection of typhoid vaccine. They, however, did not interpret their results as indicating a local formation of antibodies. We have attempted in this experiment to determine whether it is possible to produce a local formation or concentration of virucidal antibodies by repeated intradermal injection of herpes virus.

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* This research was supported by a grant from the W. J. Matheson Fund for the Study of Encephalitis.

[1] Cannon, P. R., and Sullivan, F. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 517.

[2] Seegal, B. C., and Seegal, D., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 437.

A series of rabbits were given intradermal inoculations of 1 cc. of Frank herpes virus at weekly intervals over a period of 4 to 6 weeks in the same skin area. After 10 to 40 days, the animals were exsanguinated and the inoculated area of the skin as well as some skin on the uninoculated side were excised and the tissue juice extracted following the method described by Seegal and Khorazo.[3] The fluid obtained was usually amber colored, which suggested that little blood was present. Neutralization tests were set up with varying dilutions of tissue juice and immune serum. In each case 0.4 cc. of the dilution was added to 0.4 cc. of the centrifuged supernatant virus suspension and incubated for 2 hours at 37°C. Rabbits were then injected in the cisterna magna with 0.4 cc. of the mixture. Table I is typical of numerous tests carried out.

TABLE I.
Tests for the Neutralizing Power of Immune Serum and Tissue Juice.

Dilutions	Tissue Juice Inoculated Side	Tissue Juice Uninoculated Side	Immune Serum	Normal Serum
Undiluted	Died	Died	Survived	Died
5:1	”	”	”	”
4:1	”	”	”	”
1:2	”	”	”	”
1:4	”	”	”	”
1:6	”	”	Died	”

It is obvious from our results that the tissue juice obtained from the inoculated side as well as the uninoculated side never afforded protection against herpes virus, whereas the immune serum gave protection in a dilution of 1-4. Although a general immunity has been produced, there is no evidence from our work that the local intradermal injection of virus established "a locally superior mechanism for the disposal of the particular microorganism" (Gay).[4]

Since, by histological section, we have found no increase in the number of cells present in the inoculated skin, and since we have found no virucidal action of the tissue juice *per se,* it would seem that the local immunity to subsequent skin infection is a function of the antibodies in the general circulation.

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[3] Seegal, B. C., and Khorazo, D., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 435.

[4] Gay, F. P., The Newer Knowledge of Bacteriology and Immunology, Jordan, E. O., and Falk, I. S., University of Chicago Press, 1928.

Contrary to the findings in the case of bactericidal substance, it has not been possible by the method employed to demonstrate any local formation or concentration of virucidal antibodies.

8285 P

Potential Variations of Extremities and of Precordium in Myocardial Disease.

CHARLES E. KOSSMANN. (Introduced by Arthur C. DeGraff.)

From the Department of Therapeutics, New York University College of Medicine, and the Third (New York University) Medical Division of Bellevue Hospital, New York.

Wilson, Macleod, and Barker[1] described electrocardiographic leads which record the potential variations produced by the heart beat at a single point. These leads were obtained by pairing the point in question with a central terminal connected through separate, identical, non-inductive resistances with the right arm, left arm, and left leg respectively. Further details and slight modifications were published.[2] The potential variations of the extremities and of 6 precordial points in normal subjects were studied in Wilson's laboratory.[3] In the past few years Wilson and his associates have taken numerous curves in various types of heart disease by this method, but only a few of these have been published.[4, 5]

Since July 1934, this method has been used in the study of heart disease on the wards of the Third Medical (N. Y. U.) Division of Bellevue Hospital. As in the study of normal subjects referred to[3] the electrocardiograms taken in each case were the 3 standard leads, the potentials of the 3 extremities, and the potentials of 6 precordial points. All electrocardiograms were taken with a single stage, vacuum tube amplifier in circuit with the string galvanometer.

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[1] Wilson, F. N., Macleod, A. G., and Barker, P. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1011

[2] Wilson, F. N., Johnston, F. D., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1934, **9**, 447.

[3] Kossmann, C. E., and Johnston, F. D., *Am. Heart J.*, 1935, **10**, 925.

[4] Wilson, F. N., Johnston, F. D., Hill, I. G. W., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1934, **9**, 459.

[5] Wilson, F. N., Johnston, F. D., and Barker, P. S., *Am. Heart J.*, 1934, **9**, 472.

This amplifier was designed and constructed by Dr. Franklin D. Johnston of the University of Michigan Medical School, to whom the author takes this opportunity to express his sincere appreciation. In about one-third of the cases the curves were taken simultaneously with standard lead I.

To date 183 patients have been studied. They include cases with left or right deviation of the electrical axis with or without cardiac hypertrophy; discordant and concordant left bundle branch block; typical and atypical right bundle branch block; intraventricular block; acute rheumatic myocarditis; acute fibrinous pericarditis; one case each of pericarditis with effusion and dextrocardia; and 45 cases of fresh or old infarction of the myocardium.

Thus far the method has found its greatest clinical application in the location of the conduction defect in bundle branch block, and in the diagnosis of both new and old myocardial infarcts. The alterations in the initial ventricular deflections of the precordial potentials are more characteristic when the infarct is on the anterior than when it is on the posterior wall of the heart, and are similar to those found by Wilson and his associates clinically and experimentally when the exploring electrode was paired with the left leg in humans,[6, 7] or the left hind leg in dogs.[8, 9] Temporary changes in the RS-T segment, and temporary or permanent changes in the T wave are variable, and depend on several factors, the most important of which appears to be the orientation of the exploring electrode with respect to the underlying dead and injured muscle.

Fourteen of the 183 cases studied came to autopsy. Eight of these showed infarcts of the myocardium. These hearts have been sectioned by the method used by Dr. Clarence E. De La Chapelle in the study of a large series of hearts with infarcts presently to be reported. Correlation of the precordial electrocardiographic findings with the cardiac pathology in this small group has brought out the following points with regard to myocardial infarction: (1) the greatest changes in the initial ventricular deflections are obtained when the exploring electrode is placed directly over the dead muscle; (2) in one case with extensive fibrosis of the anterior wall and a

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[6] Wilson, F. N., Barker, P. S., Macleod, A. G., and Klostermeyer, L. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1006.

[7] Wilson, F. N., Macleod, A. G., Barker, P. S., Johnston, F. D., and Klostermeyer, L. L., *Heart*, 1933, **16**, 155.

[8] Wilson, F. N., Johnston, F. D., Hill, I. G. W., and Grout, G. C., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 799.

[9] Wilson, F. N., Johnston, F. D., Hill, I. G. W., and Grout, G. C., *Trans. Assn. Am. Phys.*, 1933, **48**, 154.

relatively recent infarct in the posterior wall of the left ventricle the precordial potentials simulated those of anterior wall infarct; (3) in bundle branch block the QRS may be similar to, though wider than, that found with infarction of the anterior wall of the heart. This may easily lead to erroneous diagnosis as in one case of this series that was further complicated by marked displacement of the heart by a large aortic aneurysm. (4) Small scars in the posterior wall cannot be detected. (5) The presence of an infarct in the anterior wall may be very evident from a study of the precordial potentials when standard leads are not characteristic.

8286 P

Stimulation of Abnormal Mammary Growth by Large Amounts of Estrogenic Hormone.

W. U. GARDNER, GEORGE M. SMITH, AND LEONELL C. STRONG.
(Introduced by Edgar Allen.)

From the Department of Anatomy, Yale University School of Medicine.

Purified estrogenic hormones (theelin and theelol)* induced a growth of the mammary duct system (Turner, *et al.*[1]) Theelin stimulated a growth of the mammary duct system of male mice (Turner and Gomez[2]; Gardner, Diddle, Allen, and Strong[3]). The extent and type of development were comparable with that observed in mature virgin female mice or male mice bearing ovarian grafts (Gardner[4]).

The water soluble hormone (theelin) in daily doses of 1 to 5 rat units administered subcutaneously in 2 injections induced a localized excessive development of lobules of alveoli along the mammary ducts of male mice of one strain (C_3H). A high percentage of females of this strain developed mammary cancer. Similar lobules of alveoli have been found in the mammary glands of male mice from one other cancer susceptible and one cancer resistant strain when theelin had been administered for periods of several months.

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* ''Theelin'' and ''Theelol'' refer to the Parke, Davis Company products.

[1] Turner, C. W., *et al.*, *Anat. Rec.*, 1932, **53**, 227.

[2] Turner, C. W., and Gomez, E. T., Mo. Agr. Exp. Sta. Res. Bul. 206, 1934.

[3] Gardner, W. U., Diddle, A. W., Allen, E., and Strong, L. C., *Anat. Rec.*, 1934, **60**, 451.

[4] Gardner, W. U., *Endocrinology*, 1935. In press.

The present observations were made on male mice from 2 cancer susceptible strains (A and C_3H), which were given weekly injections of 500 international units of folliculin benzoate† in oil. The mice were from 3 to 28 days old when placed on experiment. They were removed at periods varying from 101 to 172 days. The total amounts of folliculin benzoate varied from 7,500 to 12,500 units. The 6 mice that were 28 days old developed scrotal hernias after 6 to 8 weeks of treatment. Hydronephrosis and other conditions developed at a later date resulting in a rapid emaciation.

As the mice were not removed during the early stages, the rate of mammary development was not determined. The extent and type of mammary growth will be compared with that observed in male mice receiving small daily doses of water soluble theelin.

All of the mammary rudiments did not respond. Some of them persisted as very small rudimentary ducts. The extent or area of the mammary glands was uniformly less than that in the mice receiving theelin. The major ducts were of approximately one-half the length of the controls. Unlike the theelin-treated controls, also, extensive areas of most of the glands had developed lobules of alveolar tissue. The greater part of the gland thus resembled the glands of mice during late pregnancy at which time the alveoli are well developed. Unlike the development of the glands during pregnancy, an overgrowth of the mammary connective tissue had occurred in some areas. The ducts of the glands were distended with secretion and upon examination *in toto* at low magnification presented a beaded appearance.

Microscopic examination of the extensive areas of alveolar development usually revealed well formed mammary lobules. The alveolar epithelium was frequently vacuolated and secretion was present in the lumen. Some of the alveoli were completly filled with vacuolated epithelial cells. In other lobules the epithelium was inactive and the alveoli contained but little secretion. Some lobules were in a rapid stage of development as mitotic figures were frequent. Some of the hyperplastic lobules resembled small adenomatous growths. It is believed that the cancers appearing in the glands of such mice arise in these rapidly growing areas. The distended, beaded ducts contained a more or less homogeneous secretion containing scattered epithelial cells and leucocytes. Where large amounts of secretion had accumulated the duct epithelium was very thin or absent. At intervals epithelial cells and leucocytes enveloped a section of the secretion giving the ducts the beaded appearance.

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† The folliculin benzoate [Benzo-gynoestryl, $C_6H_5CO(C_{18}H_{21}O_2)$] was obtained from Dr. Girard (Paris). It has been prepared from the urine of pregnant women and dissolved in oil (10,000 international units per cc.).

Summary. Large amounts of estrogenic hormone in the form of folliculin benzoate, when injected subcutaneously at weekly intervals, induced a stunted development of the mammary duct system as compared with that developed in mice receiving small amounts of theelin daily. Extensive areas of alveolar development and some areas of excessive development of the connective tissue stroma were induced. The extent and type of mammary growth induced in the male mice receiving folliculin benzoate thus differed from that observed in virgin female mice or induced experimentally in male mice receiving theelin.

8287 C

Paget's Disease: Relative Constancy of Serum Phosphatase Levels over Periods up to Two Years.

ALEXANDER B. GUTMAN AND ETHEL BENEDICT GUTMAN. (Introduced by M. H. Dawson.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Kay[1] and, independently, Roberts[2] were the first to point out that the phosphatase activity of the blood is increased in osteitis deformans. This observation has been confirmed and extended by subsequent investigators[3-8] whose conclusions, based upon determinations on a combined total of approximately 200 cases may be summarized as follows:

1. Increased serum (plasma) phosphatase activity is a consistent manifestation of polyostotic Paget's disease.[3-8] In localized Paget's disease, the serum phosphatase activity is usually increased[4-8] but the increase may not be marked,[4, 5, 7, 8] and in occasional cases, the values may be within normal limits.[4, 5, 8]

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[1] Kay, H. D., *Brit. J. Exp. Path.*, 1929, **10**, 253.

[2] Roberts, W. M., *Brit. J. Exp. Path.*, 1930, **11**, 90.

[3] Kay, H. D., *J. Biol. Chem.*, 1930, **89**, 249.

[4] Race, J., *Arch. Med. Hydrology*, 1932, **10**, 6.

[5] O'Reilly, T. J., and Race, J., *Quart. J. Med.*, 1932, **1**, 471.

[6] Kay, H. D., Simpson, S. L., and Riddoch, G., *Arch. Int. Med.*, 1934, **53**, 208.

[7] Bodansky, A., and Jaffe, H. L., *Arch. Int. Med.*, 1934, **54**, 88.

[8] Gutman, A. B., Tyson, T. L., and Gutman, E. B., *Arch. Int. Med.* In press.

2. There is a rough proportionality between the degree of increase in blood phosphatase activity and the extent of skeletal involvement, as shown by roentgenologic examination.[3–8] In advanced cases, the serum phosphatase may be 100 Bodansky units per 100 cc. or more, as compared with the normal range of 1 to 4 Bodansky units.[9]

3. Discrepancies in this relation may occur, however, particularly in the moderately advanced polyostotic group.[3, 5, 7, 8] Disproportionately low values may be encountered, usually in cases clinically quiescent.[5, 7, 8] Some patients exhibiting disproportionately high values have been followed long enough to demonstrate unusually rapid progress of the disease in their cases.[8] It would appear, therefore, that the level of serum phosphatase activity may reflect the rate of development of the disease in some instances, and that, in this sense, the determination may be of value in prognosis.[8]

4. The value of the determination in the detection of Paget's disease in its early stages[4, 5] is limited, since the radiologic diagnosis is often obvious before the increase in serum phosphatase is unequivocal.[8]

5. The determination is of little or no value in the differentiation of Paget's disease from osteoplastic bone metastases, particularly in carcinoma of the prostate gland, since the values obtained in this condition may be as high as any seen in advanced cases of osteitis deformans.[8]

6. Increased blood phosphatase levels are encountered in certain diseases of bone other than Paget's disease[3, 7, 10] and in some disturbances of hepatic function.[2, 11] While the determination is useful in corroborating the radiologic or clinical diagnosis of Paget's disease, it is not a specific test.

7. It is probable that the increase in blood phosphatase activity in osteitis deformans is a result and not the cause of the disease;[11] in all likelihood, it is an expression of increased cellular activities leading to bone formation.[12]

It is desired here to call attention to another aspect of the phenomenon, to which no specific reference could be found in the literature, but which provides additional evidence that the increase in

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[9] Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

[10] Kay, H. D., *Physiolog. Rev.*, 1932, **12**, 384.

[11] Bodansky, A., and Jaffe, H. L., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 107.

[12] Robison, R., The Significance of Phosphoric Esters in Metabolism, N. Y. University Press, N. Y., 1932.

TABLE I.

Paget's Disease: Relative Constancy of Serum Phosphatase Levels in 20 Cases, Followed for Periods up to 2 Years.

				Serum		
Case*	Age	Sex	Date	Phosphatase (Bodansky units per 100 cc.)	Ca mg. %	Inorg. P mg. %
1	66	♂	2-3-34	130.3	——	3.4
			9-17-35	132.4	9.4	3.2
3	65	♂	6-22-34	109.9	9.7	3.3
			3-17-35	106.9	9.7	3.5
4	60	♀	10-3-33	88.7	10.1	2.9
			10-6-33	88.1	10.3	3.1
			5-24-34	91.9	10.1	3.5
			5-28-34	96.3	——	3.2
			5-10-35	90.5	10.2	2.5
6	51	♂	9-18-34	122.2	10.7	3.2
			3-11-35	116.0	10.2	3.4
			9-17-35	123.1	10.0	3.1
8	47	♀	6-8-34	109.5	10.0	3.8
			9-18-35	123.3	10.0	3.4
9	64	♂	6-26-34	94.3	10.7	3.7
			2-27-35	60.6	9.8	2.9
			9-17-35	60.5	10.4	2.9
12	64	♀	5-14-35	78.4	10.1	3.3
			5-21-35	70.7	——	2.8
13	69	♂	6-22-34	72.6	10.7	3.0
			3-13-35	77.6	11.0	3.2
16	45	♀	6-7-34	59.6	11.7	2.8
			6-18-34	63.9	11.5	2.9
			10-11-34	58.3	11.7	3.0
			9-18-35	57.0	11.5	2.5
24	43	♂	12-29-33	47.8	10.8	3.5
			9-17-35	61.8	9.9	3.4
34	58	♀	8-29-33	36.5	10.1	3.8
			9-21-35	33.0	10.1	3.4
36	74	♂	11-23-33	33.3	9.9	3.4
			5-31-34	34.3	10.1	3.4
			9-18-35	30.0	9.8	2.6
42	63	♀	6-9-34	29.4	10.1	5.1
			7-23-34	23.3	10.9	4.2
45	75	♀	3-25-34	24.2	9.8	3.0
			10-31-34	14.6	10.1	3.5
46	55	♀	5-18-34	23.0	10.7	3.2
			10-26-34	25.8	10.5	3.6
			9-18-35	21.1	10.5	3.2
48	63	♂	8-20-34	19.7	11.2	3.2
			12-12-34	19.3	10.2	3.4
			9-20-35	18.7	10.3	2.8
56	56	♂	4-20-33	11.5	10.9	3.8
			5-6-33	13.6	10.3	3.7
58	45	♂	1-18-34	13.1	10.6	3.2
			11-13-34	12.4	10.9	2.8
			6-4-35	13.3	10.0	2.8
67	56	♂	7-10-33	6.5	13.9	2.8
			8-3-33	8.3	17.2	3.8
			8-16-33	10.9	18.9	4.5
			8-22-33	8.7	16.4	3.9
70	34	♀	12-29-33	5.3	10.8	3.4
			1-15-34	6.9	9.8	2.9
			9-18-35	7.3	9.8	2.6

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* The cases are numbered to conform with the order in Table 3, Gutman, Tyson, and Gutman[8] where the extent of bone involvement in each instance is recorded.

serum phosphatase in Paget's disease is not capricious or coincidental but significant and correlated. The data in Table I summarize the results of successive blood analyses in 20 cases of osteitis deformans,[13] the observations being made at intervals varying from several weeks to 2 years. In the majority of instances, the serum phosphatase was maintained at strikingly constant high levels. In 16 of 20 cases followed, subsequent values were within 15% of the figure obtained on the initial determination. In cases 8 and 24, there was a definite increase in serum phosphatase, in cases 9 and 45 a decrease was observed, not associated with any obvious change in the clinical picture. The serum calcium, with two exceptions, was within the range of normal variation, irrespective of the extent or stage of the Paget involvement. In case 67 (case history and necropsy findings summarized elsewhere[8]), a marked hypercalcemia was present, probably not related to the localized Paget lesion found. The serum non-protein nitrogen was essentially normal in all cases.

The phosphatase determinations were carried out in duplicate on serum from fasting patients by Bodansky's method.[9] Corrections were applied for deviations from Beer's law,[14] and for inhibition of hydrolysis by accumulation of hydrolytic products when the phosphatase activity exceeded 40 Bodansky units per 100 cc.,[15] as recommended by Bodansky.

The relative constancy of the serum phosphatase levels in Paget's disease suggests that the determination may prove useful in supplementing clinical and radiologic examination in studying the effects of various forms of therapy. It is interesting to note that in 9 of the above cases, radiotherapy was given for alleviation of pain without any apparent effect on the serum phosphatase values, whether or not the patients were benefited by treatment.

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[13] Gutman, A. B., and Kasabach, H., *Am. J. Med. Sci.* In press.

[14] Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197.

[15] Bodansky, A., and Jaffe, H. L., *Am. J. Diseases Chil.*, 1934, **48**, 1268.

8288 C

Cultivation of Vaccinia Virus by Rivers' Method.

WRAY LLOYD AND ALEXANDER F. MAHAFFY. (Introduced by J. H. Bauer.)

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There are at present two reliable methods of growing vaccinia virus in avian tissues under bacteriologically sterile conditions, namely, those of Rivers and his associates[1] and of Goodpasture and his coworkers.[2] Our experience, which was confined to the former technique, has convinced us of its extreme practicability for the cultivation of vaccinia virus. Previous reports of the successful application of this means of propagation of vaccinia virus, for use in Jennerian prophylaxis in man, have been made by Rivers,[1] Rivers and Ward,[3] Herzberg,[4] and Coffey.[5] With the exception of those of Rivers and his colleagues, none of these communications deal with prolonged cultivation of the virus.

Two strains of vaccinia virus have been grown during our cultivation experiments, one known as neurolapine (Reichgesundheitsamt) given to us by Haagen[6] after 24 mouse-brain passages; the other, Rivers' second revived cultivated strain of dermal vaccine virus after 88 subcultures in minced chicken embryo tissue and Tyrode solution.[3]

The neurolapine virus was cultivated for 30 passages in a medium of whole chicken embryonic tissue and normal monkey serum—Tyrode solution. The first series of cultures were inoculated with the supernatant fluid after centrifugalization of a 2% suspension of virus infected mouse brain in 0.9% sodium chloride solution. Each subsequent transfer was made at 4- to 7-day intervals. The infectivity of the supernatant fluid from each series of subcultures was routinely tested by the intracerebral inoculation of a group of 6-12 mice, of which number nearly all died of vaccinal meningitis 4 to 6 days following inoculation.

Rivers' second revived strain of cultivated vaccinia virus was grown for 94 subcultures during a period of 15 months without intercurrent animal passage in a medium of minced whole chicken embryo tissue and Tyrode solution. Subcultures were made at 3 or 4 daily intervals. Our serial subcultures commenced with the 88th and ended with the 182d passage. Subinoculations of culture fluid were made from culture to culture of corresponding number. This procedure rarely led to loss of the virus and ensured greatly against widespread bacterial contamination.

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[1] Rivers, T. M., *J. Exp. Med.*, 1931, **54**, 453.

[2] Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Am. J. Path.*, 1932, **8**, 271.

[3] Rivers, T. M., and Ward, S. M., *J. Exp. Med.*, 1933, **58**, 635.

[4] Herzberg, K., *Klin. Wchnschr.*, 1932, **11**, 2064.

[5] Coffey, J. M., *Am. J. Pub. Health*, 1934, **24**, 473.

[6] Haagen, E., *Zentralb. f. Bakt., Parasitenkunde u. Infektionskrankheiten*, I Orig., 1934, **131**, 420.

The potency of the virus in representative series of subcultures was determined by the intracutaneous reactions induced in rabbits from 5 to 7 days after inoculation with 0.2 cc. amounts of the decimal dilutions of the supernatant culture fluid in Tyrode solution. Although the titre varied at different times from 10^{-3} to 10^{-6}, there was no demonstrable trend to a falling titre with later passages. Such variations in apparent virus concentration as were observed from one series of subcultures to another were caused in small part by technical irregularities, but were principally due to variation in the susceptibility of individual rabbits. This deficiency was occasionally met by titrating the virus content of the culture fluid in more than one rabbit. Throughout the period of cultivation the virulence of the strain as judged by its pathogenesis for rabbit skin was well maintained and compared not unfavorably with the parent dermal strain of calf lymph origin. In all susceptible animals the intradermal inoculation of 0.2 cc. of undiluted culture fluid was followed in 2 to 5 days by the development of raised rounded lesions at the site of inoculation, with erythema of the skin, pronounced vascular congestion, slight to marked induration and peripheral dermal and subcutaneous edema. Frequently this lesion, 1.5-3 cm. in diameter, showed central hemorrhagic necrosis in dilutions of 10^{-1} and 10^{-2} and occasionally in dilutions of 10^{-3}, 10^{-4}, and 10^{-5}.

At frequent intervals during the period of cultivation, supernatant fluid from the various subcultures was diluted with an equal quantity of normal human serum and desiccated in vacuum in the frozen state. This procedure provided for the preservation of the virus at various stages of the cultivation experiment; it also offered a practical means of ensuring against loss of strain through bacterial contamination.

After dilution with an equal quantity of normal horse serum, various pools of culture fluid were desiccated in vacuum in the frozen state. Such samples when stored at 4°C. for 69 days showed an undiminished virulence for rabbits in which intradermal inoculation produced lesions in dilutions as great as 10^{-5}. The degree of preservation for a longer interval was not tested. The same lot of dried virus when subjected to a temperature of 28°C. for 28 days showed a diminution in titre for rabbit skin from 10^{-5} to 10^{-4}. Subsequent storage at 28°C. for a period of 69 days did not produce, however, a further fall in titre. The virus still produced lesions in a dilution of 10^{-4}. It has not been possible to keep the desiccated virus at undiminished titre for longer than 14 days at 37°C.

Although it was not our original purpose to test the potency of this cultivated strain of vaccinia virus in man, it is worthy of note that one previously vaccinated man inoculated intradermally with 0.2 cc. of the culture fluid from the 162d subculture developed a typical vaccinal lesion (accelerated reaction) with vesiculation, rupture of the epidermis and subcutaneous edema, induration, and ecchymosis. The lesion healed by cicatrization without secondary bacterial infection.

The possibility of the application to Jennerian prophylaxis of such a simple, practicable, bacteriologically sterile, and mammalian virus-free method as that perfected by Rivers and his coworkers is worthy of wide trial.

8289 C

Red Cell Size and Resistance to Osmotic Hemolysis.

ERIC PONDER.

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In explanation of the fact that the red cells of different mammals show different "fragilities", *i. e.*, different resistances to osmotic hemolysis, it has been pointed out that the extent dA to which the least resistant cell membrane can be stretched without there being a loss of pigment depends on the mean initial cell volume V, so that the ratio dA/V is substantially constant. This purely experimental result can be interpreted as meaning that molecules adjacent to the thin (perhaps bi-molecular) layer upon which semi-permeability depends can enter it when it is stretched, thereby allowing of a certain amount of stretching before lysis occurs (Ponder[1]).* Another well known fact is that different red cells of the *same* animal show different "fragilities", so that a resistance distribution of roughly symmetrical form arises with respect to tonicity. One would expect that here too the resistance might be related to the initial cell volume, and it is with this point that this note is concerned.

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[1] Ponder, E., *J. Physiol.*, 1935, **83**, 352.

* It is to be borne in mind that in these experiments the cells were in their spherical form. We have no information as to how the area of the red cell in its discoidal form behaves during swelling.

The volume which any cell of initial volume V_o reaches in a hypotonic solution of tonicity T is

$$V = V_o[RW(1/T - 1) + 100] \tag{1}$$

V and V_o being both measured in μ^3. In this expression W is the amount of water contained in the cell, expressed as a percentage by volume, and R is a constant which measures either the fraction of the total cell water which is "solvent water" (the view of Parpart and Shull[2]), or the extent to which the cell behaves as a "perfect osmometer" (the view of Ponder and Saslow[3]). The expression supposes that the volume of the hypotonic solution surrounding the cells is very great as compared with the volume of the cell water, as it usually is in fragility experiments. Supposing that the cell hemolyses when V reaches the critical volume V_c; then, if the stretching of the cell membrane is proportional to the initial volume V_o, we have hemolysis when

$$V_c = (0.207\ KV_o + V_o^{2/3})^{3/2} \tag{2}$$

Here the value 0.207 is that of $1/(0.75\pi)^{2/3}$. 4π, and the constant K is the constant in the expression $dA = KV_o$ which expresses the experimental result that the amount of stretching which a given cell can undergo is proportional to the initial volume V_o.

For values of K and of V_o such as are found in experiment, the right hand side of expression (2) is very nearly the same, numerically, as CV_o, where C is another constant which may assume a variety of restricted values. It therefore follows that if the cells of the same animal are distributed (roughly symmetrically) with respect to their initial volumes, both large and small cells will hemolyze in any given tonicity which produces lysis in about the same proportion as those in which they exist in the initial distribution. Thus if frequency distributions of cell volume are obtained in a number of different tonicities in which there are various degrees of hemolysis, the frequency distributions will all be of substantially the same form. This conclusion can be tested in the following way.

Frequency distributions of red cell diameters are obtained for populations of rabbit erythrocytes in isotonic plasma and in plasma sufficiently hypotonic to produce anything from 10% to 60% hemolysis, the cells being converted into their spherical form by the addition of small amounts of lecithin (see Ponder and Robinson[4]). From the distribution of diameters the distribution of volumes can easily be computed. The method used was that of Ponder and Millar,[5] the cells being photographed under the highest practicable resolution, and measurements being made from the plates. Table I shows the kind of result obtained; in this case the tonicity of the hypotonic plasma was 0.50, and the degree of hemolysis 62%. The number of cells measured for each distribution was 200, and the cells were distributed into groups differing by one division of the measuring scale, which, as the magnification was 580, was equal to 0.17μ. This is the finest grouping permissible, and relatively little is gained, in this particular case, by measuring a greater number of cells.

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[2] Parpart, A. K., and Shull, J. C., *J. Cell. and Comp. Physiol.*, 1935, **6**, 137.

[3] Ponder, E., and Saslow, G., *J. Physiol.*, 1931, **73**, 267.

TABLE I.

Tonicity = 1.0		Tonicity = 0.50	
Diameter, μ	No.	Diameter, μ	No.
4.32	6	5.00	8
4.48	14	5.17	20
4.65	40	5.35	36
4.82	60	5.51	50
5.00	42	5.70	44
5.17	26	5.86	30
5.35	8	6.04	10
5.51	4	6.21	2

For the distribution in undiluted plasma the mean cell diameter is 4.8 ± 0.018μ, with a standard deviation of 0.25 ± 0.013μ. This corresponds to a mean cell volume of $58\mu^3$. For the distribution in hypotonic plasma the mean diameter is 5.56 ± 0.019μ, with a standard deviation of 0.27 ± 0.014μ.† This corresponds to a mean cell volume of $90\mu^3$. The 2 distributions become substantially the same if every cell in the first is increased to 1.155 times its initial radius, or to about 1.5 times its initial volume, which corresponds to an area increase of from 100 to 135%. It is true, theoretically (if the hypothesis that stretching depends on initial volume is correct), that the distribution in hypotonic plasma should show a small skewness as compared with the distribution in undiluted plasma, and this is because the right hand side of expression (2) is not exactly equal to CV_0; the methods used, however, are not sufficiently accurate to detect this small skewness, for the grouping of the cells is necessarily unusually coarse, a limitation being placed on the fineness of the grouping by the fact that measurements cannot be made with a precision greater than about 0.2μ, the limit of resolution. Thus the volume distribution which can be obtained in hypotonic plasma are not capable of deciding whether it is the increase in cell volume, or the stretching of the cell membrane, which is proportional to the initial cell volume.

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[4] Ponder, E., and Robinson, E. J., *J. Physiol.*, 1934, **83**, 34.

[5] Ponder, E., and Millar, W. G., *Quart. J. Exp. Physiol.*, 1924, **14**, 67.

† The standard deviation of the cell diameters in hypotonic plasma ought, strictly speaking, to be 1.115 times that for the diameters in undiluted plasma. The apparent discrepancy is accounted for by the magnitude of the standard errors.

In the course of the investigation it occurred to me that the final result might be influenced by the fact that all the small (or large) cells of the same animal do not necessarily contain the same amount of W, nor do they all necessarily have the same value of R. I accordingly computed what would happen if the values of RW were distributed with a coefficient of variation of 20%, and if the values were uncorrelated with cell size. The computations are exceedingly tedious, but the result is the same as that stated above: as the tonicity is reduced, there appears in the volume distribution of the intact cells a small skewness which would be experimentally undetectable.

Conclusion. The experimental frequency distributions for red cell volume obtained with spherical forms in hypotonic plasma in which there are varying degrees of hemolysis are compatible either with the idea that the volume at which the cell hemolyses is proportional to its initial volume, or with the idea that the stretching of the cell membrane at the moment of lysis is proportional to the initial cell volume.

8290 C

Nature and Permeability of Grasshopper Egg Membranes. II. Chemical Composition of Membranes.*

THEODORE LOUIS JAHN. (Introduced by J. H. Bodine.)

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The secreted membranes surrounding the diapause egg of the grasshopper, *Melanoplus differentialis,* consist of 4 distinct layers: the exochorion, a thin microscopically homogeneous layer; the endochorion, an underlying granular layer; a thin (about 1.5μ) yellow cuticle; and a thick white fibrous cuticular layer.[1] These can be fur-

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* Aided by a grant from the Rockefeller Foundation for work on cellular physiology.

ther subdivided.[2] The chorion is secreted by the mother, and the cuticle is secreted by the serosa cells when the embryo is about 6-11 days old at 25°C. The only published observation on the chemical composition of the membranes is that of Campbell,[3] who obtained a positive chitosan test with the cuticle (both layers combined) but not with the chorion. Since at least 2 of these membranes are semipermeable[4] their chemical compositions are of considerable importance in interpreting data on permeability of the egg.

The chorion could be separated from the cuticle mechanically, but no method was found for separating the layers of the chorion. When eggs were soaked overnight in concentrated HCl and washed in water for several hours the white fibrous cuticle could be separated from the others in a small percentage of cases. The thin yellow cuticle was freed from the others by boiling in concentrated HCl. The solubilities and some of the chemical reactions of the layers have been investigated, and the results, together with the chemical reactions of chitin (from various sources, loc. cit.) are shown in Table I. Determination of the isoelectric point was made

TABLE I.
Chemical Reactions.

	Grasshopper membranes				
Reagent	exo-chorion	endo-chorion	yellow cuticle	white cuticle	Chitin
38% HCl, cold	sl. sol.	sl. sol.	insol.	sol.*	sol.
80% HNO_3, "	" "	" "	v. sl. sol.	v. sl. sol.	"
93% H_2SO_4, "	" "	" "	insol.	sol.*	"
38% HCl, hot	sol.	sol.	"	"	"
80% HNO_3, "	"	"	sol.	"	"
93% H_2SO_4, "	"	"	insol.	"	"
70% KOH, cold	sl. sol.	sl. sol.	"	insol.	insol.
70% KOH, 160-190° C.	sol.	sol.	ins. 15 min. sol. 90 min.	"	"
Chitosan	—	—	—	+	+
Diaphanol-Zn-Cl-I	—	—	—	—	+
Millon's	+	±	—	—	—
Liebermann's	±	+	—	—	—
Xanthoproteic	+		—	—	—
Isoelectric point	2.0 ca.	3.7 ca.	none	none	3.5

Key:
sol. = dissolved within 10 minutes.
sl. sol. = dissolved within 2 weeks.
v. sl. sol. = visible disintegration in 2 months.
insol. = not dissolved within 2 hours in hot or 2 months in cold solutions.
* = with the exception of a very thin portion which dissolved only slowly.

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[1] Slifer, E. H., and King, R. L., *J. Morph.*, 1934, **56**, 593.

[2] Slifer, E. H., unpublished observations.

[3] Campbell, F. L., *Ann. Entomol. Soc. Am.*, 1929, **22**, 401.

[4] Jahn, T. L., *J. Cell. and Comp. Physiol.*, 1935, in press.

by the prussian blue method of Loeb.[5] Pieces of chorion and cuticle were left for 24 hours in citrate buffers over the pH range 1-5 to which a few drops of weak $K_4Fe(CN)_6$ had been added. They were then washed 24 hours in buffer solution without cyanide and treated with $FeCl_3$ Exposures of only 1-2 hours in buffer-cyanide solutions as used by Yonge[5] were ineffective, probably because of the low permeability of the exochorion. Discoloration of the chorion produced by the action of $FeCl_3$ did not allow a very definite end point, so that the isoelectric point values are only approximate. Attempts to obtain a color reaction above the isoelectric point with $CuSO_4$ and $K_4Fe(CN)_6$ were unsuccessful, probably because of rather effective masking of color by the normal deep yellow of the chorion. The cuticle, however, was not masked and did not stain by either method; that is, it did not act like an ampholyte in this pH range as did the chitin of decapods in the experiments of Yonge.[5]

It can be seen from the table that the 2 layers of the chorion are probably composed of 2 proteins, the exochorion giving a positive Millon and a doubtful Liebermann reaction and the endochorion a positive Liebermann and a doubtful Millon reaction. These were observed microscopically on the combined layers. The combined layers of the chorion gave a positive xanthoproteic reaction, but low intensity of the color did not permit microscopical determination of the responsible layer. Biuret tests were all negative, probably because of the extreme insolubility of the material. The isoelectric point of the exochorion was about 2.2, while that of the endochorion was about 3.7. The value for the exochorion is one of the lowest isoelectric points known for a protein, and it agrees with the value of 2.2 postulated on the basis of electrical HCl-KCl concentration effects.[4] This agreement, although only approximate, supports the theory that modified diffusion potentials are the source of the observed concentration effects. On the basis of this theory the concentration effect with KCl should be positive above and negative below the isoelectric point of the membrane. This was found in previous experiments.[4]

The chitosan tests of Campbell[3] (confirmed by Slifer, unpublished observation) indicated that at least one of the 2 layers of the cuticle contained chitin, and one of the objects of the present investigation was to determine which of the 2 layers this was. The thin yellow layer is eliminated by its high insolubility. It remained brownish-yellow in boiling HCl and turned black but did not dissolve in concentrated H_2SO_4, and it was only slowly soluble in concentrated KOH at 160-170°C. These reactions, together with the negative chitosan and diaphanol tests and the failure to stain below pH 3.5 in the prussian blue reaction very definitely distinguished it from chitin. Lack of a high resistance to boiling HNO_3 indicates that it does not have the same chemical structure as the cuticulin of Rhodnius,[6] although it may be closely related. Failure to stain by the prussian blue method below pH 5.5 distinguished it from the cuticle of the decapods.[5]

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[5] Yonge, C. M., *Proc. Roy. Soc. London*, 1932, **B111**, 298.

The white fibrous layer of the cuticle is similar to chitin in regard to solubility except that it dissolves very slowly in cold concentrated HNO_3, a month or longer being required for a visible disintegration to occur. The van Wisselingh-Brunswik-Campbell methods for detecting chitin (KOH at 160-180° for 15 minutes followed by I_2-KI in 1% H_2SO_4 which gives a violet color, and then solution in 75% H_2SO_4 and the subsequent precipitation upon dilution of small spherical crystals, supposedly chitosan sulphate, which have affinity for acid dyes) gave positive results with this layer of the cuticle, thus confirming the observations of Campbell and of Slifer. The diaphanol-zinc-chlor-iodide method of Schulze,[7] however, never gave a positive reaction. Membranes were left in diaphanol as long as 5 weeks and were subsequently placed in zinc-chlor-iodide for one minute or longer (up to 12 hours) before washing in water, but no definite violet color was obtained with either of 2 samples of diaphanol (Grübler) used. Sometimes a salmon-red color was observed as the zinc-chlor-iodide solution was diluted, but this was always faint and never remained even for a short time when the membranes were placed in water. The acetic acid-bisulphite modification of Koch[8] also gave negative results. The nymphal exoskeleton, however, gave a positive Schulze reaction after 24-48 hours in diaphanol, thus showing that the reagents and technique were satisfactory. The composition of the white cuticle, therefore, is probably similar to but not identical with the chitin of the exoskeleton.

The nymphal exoskeleton is impregnated with proteins and gave a positive Millon reaction. The cuticular layers of the egg, however, did not give positive Millon, Liebermann, or xanthoproteic reactions.

The contradictory results obtained with these microchemical tests for chitin bring up the question of the specificity of the reactions employed. According to Campbell[3] the chitosan methods are very specific. Koch,[8] however, claims that the method of Schulze is more specific than those recommended by Campbell, and he cites the tracheae of *Apis millifera* and *Musca domestica* which gave negative chitosan but positive diaphanol-zinc-chlor-iodide reactions. The white layer of the cuticle of the grasshopper egg gives the reverse of these results. This indicates that there are probably 3 substances or chemical groupings which may be detected by either one or the other of these tests (or only 2 if they are both present in cases where both reactions are positive).

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[6] Wigglesworth, V. B., *Quart. J. Microscop. Sci.*, 1933, **76**, 269.

[7] Schulze, P., *Z. Morph. u. Ökol. Tiere*, 1924, **2**, 643.

[8] Koch, C., *Z. Morph. u. Ökol. Tiere*, 1932, **25**, 730.

8291 C

Behavior of the Spindle Fibers in Centrifuged Cells.

BASILE J. LUYET.

From the Department of Biology, St. Louis University.

As I described in a previous paper,[1] by centrifuging the cells in mitosis, in the onion root tip, for one hour with a force equivalent to 30,000 times the force of gravity, I obtained a displacement of the chromosomes in the centrifugal direction, a concentration of the cytoplasm in 2 layers at the lower portion of the cell, and a separation of the cell sap to the upper region. What happens to the mitotic spindle when the cells are treated under the same conditions is the subject of this communication. The method used is, in all its details, the same as that already described.[2]

In the spindles and spindle fibers so treated I observed the following facts: 1. Only the upper cone of the spindle is present (Fig. 1, A-E), the lower one not appearing. 2. The height of the cone often exceeds one-half, and can be as much as three-fourths, of the height of the cell (Fig. 1, B). However, all the intermediate heights between this maximum and the short cones of the prophase stage are present. 3. I never observed fibers broken or badly distorted or folded on themselves, and rarely were they entangled. They are often slightly bent, but in a smooth curve. 4. The fibers stay together as the hair of a moist tapering brush; they are never scattered in different directions. 5. The half spindle bores its way centripetally through the heavier cytoplasm. It sometimes bends under the weight of the latter and seems to turn around cytoplasmic masses in its centripetal path (Fig. 1, D).

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[1] Luyet, B. J., and Ernst, R. A., *Biodyn.*, 1934, **2**, 8.

[2] Luyet, B. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1225.

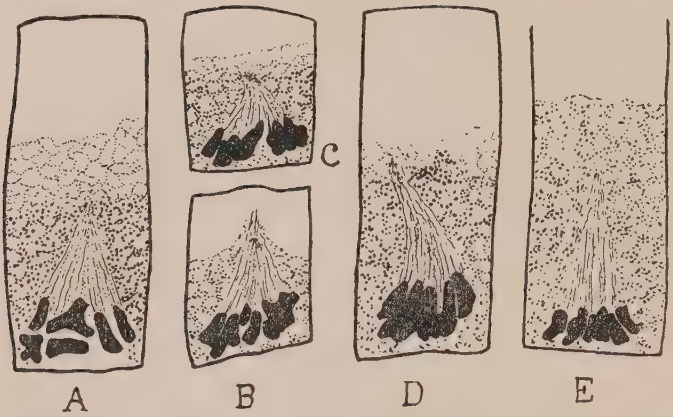

FIG. 1.

A-E camera lucida drawings of cells in mitosis, centrifuged for one hour with a force of 30,000 g. magnification : 1000 diam.

I venture the following tentative interpretation on these observations: The fact that only the upper part of the spindle appears in the picture should probably not be attributed to a crushing and destruction of the lower part, since there is usually no trace of it left; it is due rather to the centripetal displacement of the fibers of the lower cone when the heavy chromosomes to which they are attached move centrifugally.

The excess of height of the spindle might be due to a stretching of the fibers. It might be due also to the fact that the fibers located within the cone, not attached to any chromosome and not held back by any resistance, slip up under the action of the centripetal force, so that their tips protrude at the top of the cone. It is possible, as well, that during centrifugation the chromosomes slide down along the fibers to which they are attached, thus making the upper portion of the fibers longer than the lower one (suggested by Dr. G. H. Bishop).

The behavior of the spindle-fibers as described under the last 3 numbers above, seems to fit fairly well with the assumption that the fibers are structures comparable to light threads attached to the heavy chromosomes, and capable of being lifted up through the cytoplasm under the action of the centripetal force, as would threads attached by one end to stones at the bottom of a mass of water. They are held together by the unknown force which, in the untreated condition, holds them in a bundle at the pole of the mitotic spindle.

8292 P

Rate of Disappearance of Red Cells in Polythemic Rats.

DAVID B. TYLER AND FRANCIS M. BALDWIN. (Introduced by L. T. Samuels.)

From the Department of Zoology, University of Southern California.

Rats made polycythemic by prolonged exposure to oxygen lack lose a large percentage of the "excess" red cells within 48 hours following the exposure.[1]

In this experiment, rats were made polycythemic by either of 2 methods. (1) By exposure in glass chambers at an absolute atmospheric pressure of 400 mm. Hg. (2) By exposure in a large size incubator in which a suitable absorbent for CO_2 was placed, the oxygen lack being brought about by the animals themselves. In both methods, the exposure periods ranged from 2 to 15 days. The animals were supplied with sufficient food and water for the duration of the experiment.

The normal blood picture of each rat was determined before exposure. Numbered pipettes were assigned to each rat, and the usual precautions in making the blood counts were taken. Hemoglobin determinations were made after Heine and Bing.[2] Blood was obtained from the tail, excess bleeding being prevented by temporary ligation. Immediately following the exposure and at stated intervals thereafter the blood picture was again determined.

In order to ascertain the effect of more than one determination in one day, 3 blood counts were made on 11 unexposed rats. The first counts were made at 9 a. m. and considering these as normal, or 100%, the average of the second counts made 6 hours later was 96.2%. The average of the third counts one hour later was 95.5%. These rats served as "bleeding" controls.

Within a 6-hour period after the exposure almost 50% of the excess erythrocytes disappeared from circulation. Considering the count at "zero" hour as 100%, the average of the counts made 6 hours later was 88.7%. This is a significant difference as compared with the bleeding controls. The trend in hemoglobin is similar. However, absolute reliance cannot be placed on the hemoglobin changes reported owing to the uncertain accuracy of the method in a study of this nature. Yet because of the limited blood volume of the rats, this method seems most suitable at present.

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[1] Tyler, D. B., and Baldwin, F. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 823.

[2] Heine, R. W., and Bing, F. C., *J. Biol. Chem.*, 1933, **101**, 369.

The length of time it takes for the excess erythrocytes to disappear from circulation varies so greatly in each individual that too great significance cannot be attached in computing the longevity of the erythrocytes by this method.[3]

The mass disappearance of 50% of the surplus blood cells does not warrant the assumption of the so-called "frictional factor" which supposedly increases the rate of fragmentation because of the greater blood cell volume.

In certain rats a disappearance in excess of the increase takes place. No explanation can be offered for this phenomenon at present.

There seem to be 3 possible explanations for the results reported here:

1. That the excess blood may be absorbed by some organ whose function is controlled directly or indirectly by the O_2 tension of the respired air.[4]

2. That the disappearance may be due to plasma dilution or expansion to compensate for the increased viscosity of the blood.[5]

3. That the animals exposed to low atmospheric pressures are made somewhat anhydremic. This may be due either to a drop in water intake and increased water loss or an alteration of water balance between tissues and blood. Experiments are now under way to test these points.

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[3] Escobar, R. A., and Baldwin, F. M., *Am. J. Physiol.*, 1934, **107**, 249.

[4] Barcroft, J., Murray, C. D., Orahovats, D., Sands, J., and Weiss, R., *J. Physiol.*, 1925, **60**, 79.

[5] Douglas, C. G., Haldane, J. S., Henderson, Y., and Schneider, E. C., *Phil. Tr. Roy. Soc., London*, Series B, 1913, **203**, 195.

8293 C

A Bioassay of Certain Protein Supplements When Fed to Baby Chicks.*

S. F. COOK AND K. G. SCOTT.

From the Division of Physiology, University of California Medical School, Berkeley.

Several investigators have reported the occurrence of a syndrome in chicks which is similar to scurvy in appearance.[1, 2, 3] Since several different diets were used in those studies employing different protein sources, it seemed useful to compare the effects produced by various proteins when fed with the same basal ration in order to observe if other proteins allowed the same symptoms to occur as did fish meal, as reported by Holst.

The syndrome has been well described independently by Holst, McFarlane, *et al.*, by Dam, and by Schönheyder.[4] In using a ration similar to the one employed by Holst we were able to produce this syndrome which is characterized by (1) the production of hemorrhages without respect to location, (2) an anemia, and (3) a prolonged blood clotting time.

Feeding experiments. Two groups of experiments were carried on. The diets used and results were as follows:

Diet I—Control. Of 100 parts, corn 55, wheat 20, skim milk 20, NaCl 0.5, cod liver oil (Patch) 1, $Ca_3(PO_4)_2$ 1, $CaCO_3$ 1, dried yeast 1. When 21 chicks were placed upon this ration none died after 10 weeks of feeding. No hemorrhages were observed.

Diet II—Control. Essentially the same as No. I. A commercial casein was used as a protein supplement. Fifteen chicks were placed upon this diet and no hemorrhages were observed at the end of 10 weeks. The mortality was zero.

Diet III—Experimental. Of 100 parts, corn 55.5, wheat 20, fish meal (from 2 samples sold commercially) 20, NaCl 5, cod liver oil 1, $Ca_3(PO_4)_2$ 1, $CaCO_3$ 1, and dried yeast 1. When 17 chicks were placed upon this diet, the mortality was 35% in 10 weeks; all

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* We wish to express our appreciation of financial assistance rendered by the Board of Research of the University of California and of financial and material assistance rendered by the F. E. Booth Company and the California Packing Corporation.

[1] Holst, W. F., and Halbrook, E. R., *Science*, N. S., 1933, **77**, 354.

[2] McFarlane, W. D., Graham, W. R., and Hall, G. E., *J. Nutrition*, 1931, **4**, 331.

[3] Dam, H., *Biochem. Inst.*, Copenhagen, May, 1934.

[4] Schönheyder, F., *Nature*, 1935, **135**, 652.

of the birds had hemorrhages from time to time which were often fatal. When the fish meal concentration was reduced to 10% the same effect was observed.

We concluded from these experiments that the effects of feeding such fish meals were essentially different from other protein supplements here reported. The syndrome was much reduced when fish meal which had been washed with water and allowed to dry at 65°C. was used as a protein supplement in diet No. III.

A second series of experiment was planned upon a much larger scale and the protein supplements fed were commercial fish meals, types 1, 2, and 3; Commercial casein, type 4; and commercial meat scrap, type 5. The diets used in these experiments are to be found in Table I with analyses of the calcium, phosphorus, and protein content after the diets were mixed.

TABLE I.
Feed Formulas.
All diets (1-17 inclusive) contained sardine oil 1% and salt 0.5%.

Diet No.	Corn	Wheat	Casein	Calcium	Phosphorus	Yeast	Protein Supplements Type 1	Type 2	Type 3	Type 5	Protein
1	76.5	31	5.8	1.15	.67	1.25	6.25				16.4
2	75.9	31		1.13	.67	1.25	13.4				16.4
3	69.8	31		1.25	.72	1.25	20.0				19.3
4	76.8	31	5.4	1.17	.68	1.25		6.25			16.3
5	76.8	31		1.19	.66	1.25		12.5			16.5
6	71.1	31		1.13	.66	1.25		18.8			19.0
7	76.6	31	5.8	1.15	.68	1.25			6.25		16.4
8	76	31		1.11	.69	1.25			13.6		16.3
9	69.4	31		1.33	.77	1.25			20.4		18.9
10	77.1	31	11	1.25	.71	1.25					16.7
11	Same as No. 10										
12	72.2	31	16	1.19	.69	1.25					19.4
13	67.8	31		1.41	.84	1.25				22.3	19.1
14	69.8	31		1.21	.76	3.75	20.0				19.4
15	71.1	31		1.15	.69	3.75		18.75			19.0
16	69.4	31		1.29	.79	3.75			20.4		18.5
17A	69.8	31		1.17	.67	0	20.0				18.9
17B	69.8	31		1.17	.69	0		20.0			18.0

No. 18 contained Alfalfa 5, Bran 15, Wheat 27, Corn 35, Fish Meal 10, Skim Milk 5, Salt 0.5, Sardine Oil 0.5, Calcium 1.35%, Phosphorus 0.85%, and protein 17.5%.

Calcium, Phosphorus and Protein are percentages present in total mixture by analysis. Other figures are parts in 125.

Parallel experiments were carried on at 2 different laboratories for a period of 10 weeks. Fifteen chicks were placed in each group. Since the results of the 2 sets of experiments were very comparable, they are incorporated in the same table (Table II).

Results—Mortality. The mortality of the birds placed upon the diets containing fish meal types 1 and 2 (Table II) is excessively

TABLE II.
(Second Series of Experiments.)

Group No.	Protein used	% Mortality	% Hemorrhage	% Hemoglobin			Clotting Time	
				Aver.	High	Low	High min.	Low min.
1	Type 1, 5 %	3	17	48	67	25	*	1
2	" 1, 10 "	30	67	44	67	15	*	2
3	" 1, 15 "	37	80	47	69	15	*	2
4	" 2, 5 "	13	10	57	75	22	30	1
5	" 2, 10 "	43	80	49	67	20	*	1
6	" 2, 15 "	33	60	49	76	13	*	3
7	" 3, 5 "	3	3	61	84	49	12	1
8	" 3, 10 "	10	0	62	87	15	9	1
9	" 3, 15 "	0	7	57	75	13	7	1
10	" 4, 8.7 %	7	0	60	76	18	19	1
11	" " 8.7 "	7	0	62	71	47	21	1
12	" " 13 "	10	3	64	72	55	15	1
13	" 5, 18 "	3	3	55	84	30	62	1
14	" 1	20	53	50	65	20	*	2
15	" 2	24	64	50	69	9	*	2
16	" 3	3	0	63	79	49	4	1
17A	" 1	20	47	57	68	45	15	1
17B	" 2	53	46	36	52	26	33	2
18	" 1 and 2	0	0	58	75	14	4	1

* Clotting times longer than 100 min.

high with the exception of diet No. 18, and not at all comparable with the other protein supplements used.

Hemorrhages. The occurrence of hemorrhages was quantitatively much greater upon the fish meal types 1 and 2. Hemorrhages did not occur upon the other groups, including group 18. Some doubtful cases appeared upon supplements 3 and 4, but none upon supplement 5.

Hemoglobin level. When the protein level was the same, the groups fed fish meal types 1 and 2 were on the average distinctly anemic with respect to those fed other protein supplements (Sahli's method). Furthermore, in those groups fed fish meal types 1 and 2 with the same protein levels the average hemoglobin per cent was inversely proportional to the fish meal concentration (see groups 1 and 2, 4 and 5, Table II).

Blood Clotting Time. When fish meal types 1 and 2 were fed, a profound increase in blood clotting time was observed. For quantitative evidence see Table II.

Growth. The growth in all groups was comparable except in those to which no yeast was added, and was apparently normal for the stock used in the experiment.

Summary. Experimental evidence is offered to show that certain diets containing some types of fish meal induce the condition in chicks which has been described in the text. Apparently the syndrome can be ascribed to the fish meals used in that they contained objectionable materials and/or lacked some accessory factor. It was not possible to produce this syndrome quantitatively by the use of other protein supplements.

8294 P

Metabolism of Benzene, Anthracene and Phenanthrene in Adult and Growing Dogs.

JAKOB A. STEKOL.

From the Department of Chemistry, Fordham University, New York City.

In view of similarity in the metabolism of bromobenzene and naphthalene in rabbits[1] and in adult and growing dogs,[2] it seemed of interest to extend the comparison of metabolism of aromatic hydrocarbons in dogs to benzene, anthracene and phenanthrene. Each substance was fed on 4 occasions to each of the 4 growing and 2 adult dogs in 1.0 gm. doses. The pups and dogs were maintained on a diet of constant composition and the urine collected every 24 hours. The diet, the general experimental procedure, methods of analysis of urine were the same as described by us elsewhere.[3] The pups were fed benzene, anthracene and phenanthrene at the age of 2, 3, 4, and 7 months at sufficiently large intervals (7-8 days) to allow the animals to return to the original nitrogen and sulfur balance. The analysis of urine collected after feeding the hydrocarbons to pups and dogs indicated the formation of ethereal sulfates from benzene and anthracene; benzene, in addition, and especially phenanthrene, raised the output of neutral sulfur. All 3 hydrocarbons increased the output of glycuronates in the urine.

Hele[4] on the basis of experiments with benzene, similar to those described here, suggested that benzene probably yields a mercapturic acid, although the latter could not be isolated by Baumann-Preusse method.[5] Pending the isolation of detoxication products of benzene, anthracene and phenanthrene from the urine of dogs and pups, now in progress, it is assumed that benzene and phenanthrene are conjugated in the animal body to yield, perhaps, a mercapturic acid type product, similar to p-halogen-phenylmercapturic and l-α-naphthalene mercapturic acids.

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[1] Bourne, M. C., and Young, L., *Biochem. J.*, 1934, **28**, 803; Nakashima, T., *J. Biochem.* (Japan), 1934, **19**, 281.

[2] Stekol, J. A., *J. Biol. Chem.*, 1935, **110**, 463.

[3] Stekol, J. A., *J. Biol. Chem.*, 1934, **107**, 641; 1935, **109**, 147.

[4] Callow, E. H., and Hele, T. S., 1926, **20**, 598.

[5] Baumann, E., and Preusse, C., *Ber. deutsch. chem. Ges.*, 1879, **12**, 806.

8295 C

Effects of Various Anesthesias on Autoxidation Rate of Surviving Brain Tissue.

GEORGE A. EMERSON. (Introduced by Paul D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, Tenn.

It has previously been shown that ether anesthesia in rats brings about a condition, demonstrable in brain tissue removed immediately after termination of one hour of deep surgical anesthesia, such that the rate of autoxidation of available carbohydrate and lactic acid in the surviving brain decreases more rapidly than is usual for tissues taken from untreated rats.[1] Since both the total carbohydrate and glycogen content of rat brain decreases in ether anesthesia[2] despite the marked hyperglycemia maintained at the same time, it was suggested that the inhibitory effect on rate of autoxidation was due simply to limiting of oxidizable carbohydrate in the excised tissue. The ability of such tissue to metabolize added glucose at a normal rate substantiates this.

Examination of data on blood and urine chemistry shows marked resemblance of biochemical effects of epinephrine and ether. The possibility that many of the physiological side actions of ether are mediated through stimulation of adrenin output is supported by the weight of much evidence,[3] and studies on intestinal chemistry[4] and ether ketosis[5] show marked parallelism of many effects of the 2 agents. Unequivocal direct evidence of the supposed action of ether on the suprarenals is as yet lacking.[6] The present communication is concerned with demonstrating whether other narcotics having some sympathomimetic actions in the organism effect changes in rate of autoxidation of surviving brain tissues taken from anesthetized rats.

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[1] Emerson, G. A., *J. Tenn. Acad. Sci.* In press.

[2] Uchida, S., *Biochem. Z.*, 1926, **167**, 9.

[3] Knoefel, P. K., *California and Western Med.*, 1933, **39**, 5.

[4] Emerson, G. A. To be published.

[5] Emerson, G. A., *J. Pharm. Exp. Therap.*, 1935, **54**, 90.

[6] Elliott, T. R., *J. Physiol.*, 1912, **44**, 374; Schlossmann, H., and Mügge, H., *Arch. Exp. Pathol. Pharmacol.*, 1929, **144**, 133; Fujii, I., *Tohoku J. Exp. Med.*, 1925, **5**, 566; Kodama, S., *Tohoku J. Exp. Med.*, 1924, **4**, 601.

Five to 10 individual experiments were carried out with each agent tested, and single typical responses selected for Table I. The direct method[7] of measuring respiration in a Warburg apparatus was used. Consequently there was lacking the same check on the disturbing influence of possible variations in the partial pressures of foreign anesthetic gases as was easily obtained[1] in the indirect method of Warburg by examining the magnitude of CO_2 production in excess of theory. However, since the present results agree well for the case of ether with those previously obtained by the indirect method, it may be assumed for the present purposes that the results with other agents are likewise real and not artefacts due to either liberation or absorption of the anesthetic gas by the fatty brain tissue during the course of measurement of respiration.

TABLE I.
Oxygen Consumption of Surviving Brain Tissue from Anesthetized Rats During Autoxidation.

	$-Q_{O_2}$*					
	Time of Autoxidation, Min.					After Glucose, 0.2%
Anesthetic Agent	15	30	45	60	75	30
Ether	9.4	4.6	3.8	2.6	2.2	8.2
Divinyl Oxide	7.4	7.2	5.0	4.2	3.6	5.8
Ethylene	6.8	6.76	5.64	4.6	4.4	6.7
Cyclopropane	7.0	5.2	4.4	4.0	3.7	5.06
Nitrous Oxide	8.2	7.2	6.24	4.16	3.6	8.0
Ethanol	10.6	8.4	5.8	5.0	4.8	8.2
Dihydromorphinone	7.1	5.7	4.9	4.6	4.4	6.3
Chloroform	8.4	5.1	4.7	3.3	3.1	7.4
Epinephrine	7.4	5.2	4.6	3.6	2.4	7.2
Control	8.2	7.1	6.4	5.8	5.2	7.8

*$-Q_{O_2}$ expressed as mm.^{3} of O_2 absorbed per hour per mg. dry weight of tissue; calculated for each 15-minute interval terminating at the time above.

Rats were anesthetized for one hour by placing them in an 18 l. chamber initially containing 2.5 mM/l. of ether, 2.0 mM/l. of divinyl oxide or 0.8 mM/l. of chloroform in oxygen, the mixtures being made up by Fühner's method.[8] For the anesthetic gases, mixtures with oxygen were made up by using a flow-meter apparatus, and were passed through a 10 l. chamber in which the rats were placed for 1 hour. The following concentrations were employed:

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[7] Dixon, M., and Elliott, K. A. C., *Biochem. J.*, 1930, **24**, 820.

[8] Fühner, H., *Biochem. Z.*, 1921, **115**, 235.

ethylene, 85%; cyclopropane, 20%; and nitrous oxide, 90% in oxygen. Five ml./kg. of ethyl alcohol, in a 40% aqueous solution, were injected intraperitoneally in 5 rats and 0.5 mg./kg. of epinephrine hydrochloride or 5.0 mg./kg. of dihydromorphinone hydrochloride (Dilaudid, N. N. R.) subcutaneously in 10 rats.

The whole brain of each anesthetized rat was quickly excised, minced and a 50 to 100 mg. aliquot weighed to the nearest 0.5 mg. at the end of an hour after beginning administration of the various agents. The weighed tissue was quantitatively transferred to a Warburg manometer vessel containing 3.0 ml. of a balanced physiological salt solution at pH 7.4 and equilibrated with oxygen for 10 minutes at 37° before commencement of measuring respiration. The entire process from the time of excising the brain tissue to beginning respiration measurements thus occupied about 15 minutes. At the end of one and a quarter hours of autoxidation, glucose was tipped from a side pocket in the manometer vessel into the menstruum about the tissue to make a final concentration of 0.2%, and respiration followed for another 30 minutes. Control tissues from the brains of untreated rats were included in each experimental run, and duplicate aliquots taken as checks in about half the cases. The pH of the contents of the manometer vessels was checked in all cases at the end of the experiment.

Quastel and Wheatley[9] have shown that while the initial autoxidative rate of brain tissues from unselected animals of any species may vary over a wide range, comparable results on different brains may be obtained if the tissues are exhausted by autoxidation until the reserves of glucose and lactate are depleted, whereupon addition of available substrate produces fairly uniform responses. This implies that the activity of the dehydrogenase systems in normal brain tissue is also reasonably uniform, and that the initial autoxidation rate is dependent on the amount of available carbohydrate present at the moment, while the decrease in autoxidation rate is a measure of the amount of reserve carbohydrate. Thus in the present work it was found that the initial autoxidative rate was not significant and may vary from 6.5 to 10.5 mm^3. of O_2 per hour per mg. of dry weight of tissue, while the rate of decline of autoxidation is sufficiently constant in different brain tissues taken from untreated fed rats to permit significant comparison with experimentally treated animals. Such results are included in Table I. In order to magnify the decrease in autoxidative rate, oxygen consumptions expressed as $-Q_{O_2}$ over single consecutive 15-minute intervals are given rather than the summated $-Q_{O_2}$ for the entire time period, as is customary.

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[9] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1932, **26**, 725.

The present results should not be taken to indicate direct effects of the narcotics studied upon oxidative processes in the brain. As has been pointed out by Quastel and Wheatley,[10] such studies should be done with tissue taken from narcotized animals which is subsequently allowed to oxidize in media containing approximately that concentration of the narcotic agent which may be found in sera of deeply narcotized animals. Even with agents highly soluble in lipoids, the concentration is effectively reduced by placing the tissue in 30 to 60 volumes of aqueous media, as is done in the present experiments. The positive effects here noted are better interpreted as being due to biochemical changes in the tissue not directly related to the presence of low concentrations of narcotics in the brain; the one exception to this may be in the case of ethanol.

Ether and chloroform anesthesias and massive injection of epinephrine hydrochloride were found to inhibit the autoxidative rate significantly (Table I). If results in all the experiments are to be interpreted as modified by sympathetic activity, the slight effects with nitrous oxide might be referred to that dependent on the slight anoxemia present; with divinyl oxide, to a slight direct sympathetic effect or through anoxemia brought about by the primary increased flow of mucus and resultant faulty gas exchange; and with cyclopropane to a relatively small direct effect on the sympathetic nervous system.

Results with ethanol confirm those of Robertson and Stewart[11] and Wortis,[12] although the extra uptake of O_2 cannot be considered very great. Dilaudid has apparently less activity on the autoxidative rate than might be expected from its hyperglycemic action; with morphine, Gross and Pierce[13] have reported marked increases in autoxidative rates of brain tissue from non-tolerant rats receiving 50 mg. of morphine sulphate per 100 gm., above the lethal dose. In view of the decrease in total carbohydrate content of the brain after morphine, reported by Uchida,[2] this suggests that other biochemical mechanisms than the simple limiting of substrate or enzyme system are involved. The findings of increased autoxidative rate[12] in tissues from rats receiving massive doses of glucose + a small dose of epinephrine insufficient to interfere with carbohydrate metabolism, do not contradict the present results showing a decrease after administration of a massive dose of epinephrine without glucose. Especially applicable to the explanation of the present findings are the data of Wortis[12] illustrating marked depression of autoxidation in rats fasted 2 to 3 days and given insulin.

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[10] Quastel, J. H., and Wheatley, A. H. M., *Proc. Roy. Soc., B*, 1932, **112**, 60.

[11] Robertson, J. D., and Stewart, C. P., *Biochem. J.*, 1932, **26**, 65.

[12] Wortis, S. B., *Arch. Neurol. Psychiat.*, 1935, **33**, 1022.

[13] Gross, E. G., and Pierce, I. H., *J. Pharm. Exp. Therap.*, 1935, **53**, 156.

The inconstancy of the normal glycogen content of brain tissue of rabbits found by Holmes and Holmes[14] doubtless has some influence on the constancy of control rates of brain autoxidation. The data of Uchida[2] show regular decreases of glycogen and total carbohydrate content of rat brain after administration of various narcotics, however, and these are reflected in results on autoxidation rates if a sufficiently large series of rats are used. Variations in the concentrations of lactic acid or other oxidizable substrates are not considered here.

Table I illustrates that the effect of anesthesia with various narcotics on the rate of autoxidation of surviving brain differs markedly with the chemical nature of the narcotic, as the depth of anesthesia was designed to be approximately equivalent for all cases. It is known that the agents studied differ markedly in their glycogenolytic potencies, and on the assumption that some correlation might be obtained between this and the autoxidative rate changes, the effect of these agents on blood sugar during narcosis was studied. As the brain respiration studies were carried out on fed male rats, glucose determinations were similarly done on fed rats from the same source, though less consistent results were so obtained than if the rats had been fasted as in previous work on glucose tolerance in normal and leprous rats.[15] After 2 control samples of blood had been drawn intracardially from rats at 10-minute intervals, the rats were treated with the same concentrations of anesthetic agents as previously for one hour. Heart blood was obtained at 10-minute intervals from each rat during the time of anesthesia and the glucose content estimated by the Hagedorn-Jensen[16] micro-method. The results of some 260 determinations are summarized in Table II.

As in other species, ether and chloroform anesthesias and epinephrine bring about a marked hyperglycemia in rats, cyclopropane and nitrous oxide a smaller rise in blood sugar, and ethylene and the necessary experimental technique none. Divinyl oxide anesthesia apparently causes a very sudden increase which is not sustained; this may be due to both a slight direct effect and an anoxemia

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[14] Holmes, B. E., and Holmes, E. G., *Biochem. J.*, 1925, **19**, 492, 836; 1926, **20**, 1196.

[15] Emerson, G. A. To be published.

[16] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46; **137**, 92.

TABLE II.
Comparative Action of Anesthetics on Blood Sugar of Fed Rats.
Results Expressed as mg. % Glucose.

Treatment	No. Rats	Preanesthetic Controls		Time of Anesthesia, Min. 10	20	30	40	50	60
Diethyl ether, 2.5 mM/l.	6	124	124	167	203	212	224	226	235
Divinyl oxide, 2.0 mM/l.	5	109	113	198	183	163	153	150	132
Ethylene, 85% in O_2	3	111	109	115	109	109	111	114	114
Cyclopropane, 20% in O_2	4	114	113	145	127	123	145	141	130
Nitrous oxide, 90% in O_2	3	124	118	137	151	149	172	159	169
Chloroform, 0.8 mM/l.	5	121	124	145	162	182	192	191	197
Epinephrine Hydrochloride 0.5 mg./Kg., subc.	5	126	124	173	216	233	270	282	279
Untreated Controls	2	115	108	107	112	116	111	109	112

effected by the marked salivation which occurs only during induction with this agent. The absence of a sustained marked hyperglycemia in deep surgical anesthesia with divinyl oxide confirms experimental work of Phatak[17] on larger mammals. With the small series of rats studied here, the standard deviations of the groups are high; this value varies in the different groups, for the glucose determinations taken after 30 minutes of anesthesia, from ±2 mg. % for ethylene to ±13 mg. % for ether and ±14 mg. % for chloroform.

Isoamylethylbarbituric acid (Amytal, N. N. R.) may inhibit glycogenolysis normally occurring in ether anesthesia[18] in which no preanesthetic medication has been given, as it does in the case of other glycogenolytic narcotics.[19] Amytal was found to inhibit the effect of ether anesthesia on autoxidation of surviving brain tissue as well. (Table III.) Three rats were anesthetized with 2.0 mM/1. of ether for 30 minutes; of these, 2 had been given 20 mg./kg. of amytal 15 minutes before induction of anesthesia. Since amytal alone has no significant action on autoxidation,[12] the results demon-

TABLE III.
Single Experiment Illustrating the Effect of Amytal Premedication on Ether Inhibition of Autoxidation in Surviving Brain.

Treatment	$-Q_{O_2}$ Time of Autoxidation, Min. 15	30	45	60	75	After Glucose, 0.2% 30
Ether, 2.0 mM/l.	7.40	5.70	4.20	3.50	3.10	6.70
Ether + Amytal	6.40	5.86	5.60	5.24	4.90	6.00
Untreated	7.60	6.40	6.10	6.00	5.80	6.80

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[17] Phatak, N. M., unpublished data.

[18] Knoefel, P. K., *J. Indiana State Medical Assn.*, 1935, **28**, 217.

[19] Olmsted, J. M. D., and Giragossintz, G., *J. Lab. Clin. Med.*, 1931, **16**, 354.

strate the dependence of the autoxidative rate upon glycogenolysis during anesthesia.

Summary. The present findings of decreased autoxidative rates in brains of rats anesthetized with glycogenolytic narcotics or treated with epinephrine are in accordance with the concept of general mediation of many biochemical processes during anesthesia through stimulation of adrenin output, with the possible exception of Dilaudid. In other cases studied, blood sugar curves show an inverse relationship to the rate of autoxidation after administration of narcotics, and no direct correlation with the depth of anesthesia produced by different anesthetics.

8296 C

Influence of Acid Extract of Cattle Anterior Pituitary Gland on Bone Repair in Young Guinea Pigs.

MARTIN SILBERBERG AND RUTH SILBERBERG. (Introduced by Leo Loeb.)

From the Department of Pathology, Dalhousie University, Halifax, N. S.

We have shown[1] that a growth-promoting effect is exerted by acid extract of cattle anterior pituitary gland on bone and cartilage in young guinea pigs. The present investigation deals with the influence of this extract on callus formation and healing of fractured bones.

Twenty-four fall and winter guinea pigs averaging from 130 to 300 gm. in weight were used in these experiments. Under deep ether anesthesia both tibia and fibula of the left hind leg as a rule were fractured in these animals. As exposure of the bone with the unavoidable removal of the periosteum was followed by unsatisfactory healing, the following procedure was used: After shaving the skin of the leg the bones were broken at about the middle of their shafts. The broken ends were carefully approximated, the leg fixed with a small wooden splint and tightly bandaged with adhesive plaster. Sixteen animals thus operated on were injected with from 1 to 1½ cc. of extract daily, while 8 others of similar size, weight and age were not injected and kept under the same environmental factors, thus serving as controls. All were fed in the same manner with oats and bran or shorts. In addition, they received some cabbage, hay, lettuce and turnips. The injections were continued for periods varying between 6 and 21 days. After 6, 10, 14, and 21 days X-ray pictures were taken in collaboration with Dr. S. R. Johnston in order to observe the position of the fragments and the progress of callus formation.

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[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423.

After 6, 10, 14, and 21 days the animals were sacrificed, and the fractured bones used for microscopical study. In each case the long bones were taken out as a whole, the muscles and tendons removed and *in toto* sections prepared, as described previously.

After the first injections the guinea pigs lost weight, but about 8 days later the original weight had been regained. The control animals gained weight steadily. In each case we compared control and injected guinea pigs in which the position of the fragments and their displacement, if there was any present, were as similar as possible.

The *gross appearance* revealed the fact that the callus formed in the injected animals was invariably harder, denser and more abundant than that of the controls as early as after 6 days, but still more pronouncedly after 10 and 14 days. After 21 days a complete, firm consolidation had taken place in the injected guinea pigs. In the majority of cases the callus had become denser and subsequently smaller in the injected as compared with the control animals.

In addition, the process of decalcification was very much more retarded in the former than in the latter guinea pigs in spite of their similarity in weight, size and age.

The *histological examination* of the callus in the controls 6 days after the fracture shows that the broken ends have become necrotic and completely calcified. They are surrounded by newly formed osteoid tissue originating from the well preserved periosteum. However, a complete union has not yet been obtained. The stumps are still separated by a layer of fresh vascularized loose granulation tissue. There is no evidence of cartilage formation.

The injected animals, at that time, show a complete union of the very well calcified stumps by means of an extensive layer of well developed, newly formed, though not yet calcified osteoid tissue. In addition, a large number of osteoblasts and of islands of vesicular cartilage has been produced. These cartilage cells show a basophilic hypertrophic cytoplasm and very frequent mitotic figures.

Ten days after the fracture the callus of the control animals consists of a large and dense layer of homogeneous vesicular cartilage cells with a very light, somewhat acidophilic cytoplasm. Complete union of the bony fragments by cartilagenous and osteoid tissue is definite at this period. In the animals which had received 10 injections of acid extract the organization of the callus is still further advanced. The callus is very much ossified. Pronounced calcification of the hypertrophic and hyperplastic vesicular cartilage cells has taken place; also the osteoid tissue has become calcified. In the control animals, as a rule, this calcification is not observed until 16 to 18 days following the fracture.

The source of the calcium in these processes, whether from the food or from parts of the skeleton, remains as yet uncertain.

After 3 weeks the callus of the control guinea pigs consists of true bone with remnants of not yet calcified osteoid tissue with the admixture of more or less cartilage. Absorption of bone by osteoclasts as well as by the blood runs parallel with the formative processes.

Within 2 to 3 weeks the absorption of the abundant callus by osteoclasts as well as by blood has become more active in the injected than in the control guinea pigs. After 3 weeks only a densely calcified small bony callus is found.

It may be mentioned that these effects are observed independently of the position of the fragments; furthermore they are the same irrespective of the sex of the animals.

However, in about 20% of the cases the process of calcification is retarded in the injected guinea pigs. Under this condition only an increased growth of hypertrophic vesicular or basophilic cartilage cells with a large number of mitotic figures is noticeable. This is observed without exception in those animals where the closure of the epiphyseal line was not found after the average number of injections.

Conclusions. Acid extract of cattle anterior pituitary gland has a growth-promoting effect on the callus formation of fractured bones in young guinea pigs. The healing tendency of fractures is accelerated. This is due to a faster transformation of mesenchymal granulation tissue which is converted

(1) directly into osteoblasts, osteoid tissue and bony substance or

(2) indirectly into cartilage and afterwards into bone by way of endochondral ossification.

Associated with the increase in formative processes there takes place also a more active absorption of the newly formed tissue.

There is noted a parallelism between the formation of cartilage and the rapidity of calcification of the callus on the one hand and the rapidity of endochondral ossification in the epiphyseal line on the other hand.

8297 P

Immunological Properties of the Smegma Bacillus in Experimental Tuberculosis in Rabbits.*

EUGENE DE SAVITSCH, KENNETH L. BURT, AND MOLLY H. RADFORD.
(Introduced by A. J. Carlson.)

From the Departments of Physiology and Pathology, University of Chicago.

Sewall and de Savitsch[1, 2] have previously demonstrated a "biological antagonism" between the so-called smegma bacillus† and the tubercle bacillus. The present work was undertaken in the attempt to apply these findings to the problem of defense of the lungs against tuberculous infections.

Twenty-four male snuffle-free rabbits of uniform weight, averaging 2,500 gm., were divided into 3 groups of 8 animals each which were treated as follows: Group A received 4 intratracheal inoculations of 10 mg. each of young viable smegma bacilli at 2-day intervals. Twelve days following the last inoculation 2.5 mg. of a 22-day-old culture of virulent bovine tubercle bacilli‡ were given to each animal by the same route. (.01 mg. of this strain constitutes a lethal dose.) In each case the bacilli were suspended in 1 cc. of physiological salt solution. To avoid the possibility of the bacilli getting into the stomach instead of the lungs a larger catheter was introduced first into the esophagus. Groups B and C, the control groups, received smegma bacilli and tubercle bacilli respectively.

The results were evaluated on the basis of weekly weight curves, longevity, and pathological involvement. Autopsies were performed soon after death and the degree of tuberculous involvement was estimated macroscopically. Histological examination was carried out upon Zenker-fixed celloidin-embedded sections stained with hema-

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* We wish to express our appreciation for the cooperation and advice of Professor Anton J. Carlson, Professor Paul C. Cannon, and Dr. Mercy Southwick. Indebtedness is acknowledged to Mrs. Virginia King of the Department of Pathology, and Miss Natalie Tupikova and Mr. Nathan Brewer, of the Department of Physiology, for their technical assistance.

[1] Sewall, H., and de Savitsch, E., Trans. 24th Ann. Meeting Nat. Tuberculosis Assn., 1928, 234.

[2] Sewall, H., and de Savitsch, E., Trans. 25th Ann. Meeting Nat. Tuberculosis Assn., 1929, 166.

† The original culture was obtained through the courtesy of Dr. G. C. Lake of the Hygienic Laboratory at Washington, D. C.

‡ We are indebted to Dr. H. J. Corper of the National Jewish Hospital in Denver for the culture of tubercle bacilli.

toxylin and eosin and upon a duplicate series stained by van Gieson's method for connective tissue. The lungs, liver and spleen were examined with reference to the extent of tuberculous involvement and to the type of lesions. The largest weight gain was shown by the animals in the smegma control group. The immunized group gained weight up to the 60th day, going down until practically the original weight was reached on the 260th day. The tuberculous controls with the exception of the first 20 days showed a sharp drop, the surviving animals retaining but 50% of their original weight on the 140th day.

The average life span of the tuberculous controls was 66 days as compared with 175 days plus for the experimental group of which 2 are still alive at the time of this writing. All rabbits in the smegma control group survived the period of the experiment. At the end of 290 days representative animals were sacrificed for post mortem study. No lesions could be demonstrated on either gross or microscopic examination.

On post mortem examination all of the animals in both the experimental and tuberculous control groups showed massive pulmonary tuberculosis. On microscopic study, however, the animals in which smegma bacilli preceded the inoculation with tubercle bacilli showed less caseation and more fibrosis in the lungs than did the rabbits receiving tubercle bacilli alone. The incidence of tuberculous involvement in the liver and spleen was rare. The liver was involved in 3 animals of the experimental group whereas no lesions were found in the tuberculous controls. The spleen was involved in only one animal of each group. No lesion could be demonstrated in any of the kidneys.

The authors believe that artificial immunity to tuberculosis is best induced, not by the tubercle bacillus itself, or by any of its fractions, but by a living non-pathogenic acid fast organism which is closely related to it. These requirements are admirably fulfilled by the smegma bacillus.

8298 C

Effect of Terminal Procedures on Liver Glycogen.

WILLIAM F. REINDOLLAR. (Introduced by John C. Krantz, Jr.)

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The purpose of this investigation is twofold: (a) to ascertain the effect, if any, of 2 anesthetics of the barbital series containing the cyclohexenyl group, upon the liver-glycogen content of the fasting white rat; (b) to study any possible chemotherapeutic effect that may be attributed to a methyl group linked directly to the nitrogen in one of these compounds. The 3 compounds given consideration were

(1) N-methyl 5,5′ methyl, Δ′ cyclohexenylmalonylurea—Evipal.
(2) N-desmethyl 5,5′ methyl, Δ′ cyclohexenylmalonylurea.
(3) N-desmethyl 5,5′ ethyl, Δ′ cyclohexenylmalonylurea—Phanodorn.*

Numbers 1 and 2 are identical except for the absence of a methyl group linked to the nitrogen in the latter case; numbers 2 and 3 are identical except that in phanodorn an ethyl group replaces the methyl group. Compounds 2 and 3 are identical in hypnotic activity, furthermore compound number 2 is unstable; hence numbers 1 and 3 were chosen for investigation.[1]

Male albino rats weighing between 100 and 150 gm. were fed at least 5 days on a standard diet and then fasted 48 hours before using. Those of the first group were killed by decapitation, the liver was extirpated and its glycogen content determined. The second and third groups were anesthetized with sodium evipal and sodium phanodorn respectively; the liver being removed as soon as surgical anesthesia was obtained.

Glycogen was determined by first extracting and converting to glucose, using Good's[2] modification of the Pflüger[3] method and titrating the glucose, employing the technique of Shaffer and Somogyi.[4]

In order to obtain a measure of the degree of precision of the method it was applied to aliquots of fresh beef liver previously digested in 30% potassium hydroxide solution made up to a definite volume; also to the pooled livers of 5 fasted rats similarly treated. The results are recorded in Table I.

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* The phanodorn used in these experiments was furnished through the kindness of the Winthrop Chemical Co.

[1] Personal communication: Charles W. Hooker, Winthrop Chemical Co.

[2] Good, Kramer, and Somogyi, *J. Biol. Chem.*, 1933, **100**, 485.

[3] Pflüger, *Pflüger's Arch.*, 1906, **114**, 242.

[4] Shaffer and Somogyi, *J. Biol. Chem.*, 1933, **100**, 695.

TABLE I.

Material	Determinations No.	Min. %	Max. %	Mean %
Fresh Beef Liver	6	0.97	1.09	1.06
Pooled Rats' Livers	5	0.03	0.04	0.03

Table II combines the results on the fasted animals killed by decapitation, anesthetized by sodium evipal, and sodium phanodorn respectively.

TABLE II.

Terminal Method	Animals No.	Glycogen, % Min.	Glycogen, % Max.	Glycogen, % Mean	Probable Error of Single Determination	Probable Error of Mean
Decapitation	30	0.03	0.44	0.14	±0.07	±0.02
Sodium Evipal	32	0.04	0.60	0.23	±0.10	±0.03
Sodium Phanodorn	35	0.03	0.45	0.16	±0.08	±0.02

Summary. A comparison of the effect of 2 methods of terminal procedure, decapitation and anesthesia, upon the liver-glycogen content of the albino rat has been made. Neither of the 2 drugs employed depressed the liver-glycogen content; neither was significantly glycogen sparing.

8299 C

Effect of Prolonged Exercise on Polynuclear Count in Man.

J. J. BOOKMAN AND D. J. FRAAD. (Introduced by E. Ponder.)

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It is generally agreed that the white cell count in man increases to a considerable extent after severe exercise of short duration. This increase, however, does not involve any appreciable shift in the polynuclear count and is accordingly to be regarded as due to a redistribution of white cells from "organ reservoirs" rather than as the result of marrow activity.[1] Hitherto no attempt has been made to find the effect of prolonged exercise on the polynuclear count, and it is with this point that this note is concerned.

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[1] Ponder, Saslow, and Schweizer, *Quart. J. Exp. Physiol.*, 1932, **22**, 21.

The experiment was carried out on 14 members of the Brown University football team, all in excellent physical condition, and all between the ages of 19 and 23. The first counts were made before the team went into training, and none of the subjects had been engaged in severe exercise for months previously. The "prolonged exercise" consisted of the usual 3-hour period 6 days a week of training and practice, including the weekly football game, and this continued from September 10th to November 24th. Counts were made at approximately weekly intervals throughout the training period. One hundred polymorphs were counted in each case, and the Cooke and Ponder criterion was used throughout.[2] All the counts were made by the same observer, the accuracy of whose counting had been previously ascertained.

In no case did the polynuclear count show any significant alteration during the period of "prolonged exercise". Initially, the weighted means of the individual counts all lay within the range 2.17-2.38, *i. e.,* within the normal range for this part of the country,[3] and in no case was there any deviation greater than that which might be due to errors in counting. The period of prolonged exercise and training accordingly had no effect on the polynuclear counts of the subjects. This is rather remarkable in view of the fact that the total white cell count during football games, etc., must have fluctuated violently; the activity of the marrow, however, as revealed by the figures for the polynuclear count, appears to have been remarkably steady.

Conclusion. The polynuclear count in man is not significantly affected by prolonged severe exercise, such as football practice of 10 weeks' duration.

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[2] Cooke, W. E., and Ponder, E., "The Polynuclear Count," London, 1927.

[3] Macleod, J., *Am. J. Physiol.,* 1935, **111**, 654.

8300 C

A Cultural Method for Classifying Staphylococci as of the "Food Poisoning" Type.

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Staphylococci isolated from sporadic cases of food poisoning are a problem in any health department since so much is dependent upon feeding tests for experimental proof of their etiological significance. Often such evidence is lacking or difficult to obtain. Los Angeles County has experienced difficulty with this problem many times. Conditions, therefore, favored the gathering of a representative collection of strains of staphylococci. Investigation of these strains has led to the development of a valuable cultural test which separates enterotoxic strains from the non-enterotoxic within the collection. So far as we know, this is the first time that a cultural characteristic common to the enterotoxic strains has been demonstrated.

Owen[1] isolated staphylococci from dried beef which had affected 19 persons. Twenty-eight years later, the American Medical Association[2] published an editorial on staphylococcus food poisoning that dealt with the successful feeding tests made in human beings by Dack, Cary, Woolpert, Oram and Wiggers.[3] This test was a revival of the one used by Barber,[4] who suffered gastro-enteritis after drinking a culture of staphylococcus. Jordan and his coworkers[5] confirmed the results of feeding tests made in human beings and with McBroom[6] introduced feeding tests conducted in monkeys. Woolpert and Dack[7] described tests in monkeys that seemed to be more successful than those conducted by Meyer[8] and ourselves. Other work, including that of Dolman[9] warrants the conclusion that the reaction of human beings is the most dependable physiological test of food poisoning. Meyer[8] mentions the fact that alarming symptoms have developed in human volunteers. Therefore, for various reasons routine human tests are impractical.

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[1] Owen, *Physician and Surgeon*, 1907, **29**, 289.

[2] Editorial, *J. A. M. A.*, April 13, 1935.

[3] Dack, Cary, Woolpert, Orem, and Wiggers, *J. Prev. Med.*, 1930, **4**, 167.

[4] Barber, *Phil. J. Sc., Sect. B*, 1914, **9**, 515.

[5] Jordan and co-workers, *J. A. M. A.*, 1930, **94**, 1648.

[6] McBroom, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 161.

[7] Woolpert and Dack, *J. Inf. Dis.*, 1933, **52**, 6.

[8] Meyer, Sonderabdruck aus Zangger-Festschrift Seite 278-289, 1934, Rascher and Cie, A.-G. Verlag, Zurich.

[9] Dolman, *J. Inf. Dis.*, 1934, **55**, 172.

Since the report of Dack, *et al.*, we had isolated staphylococci from sporadic outbreaks of food poisoning involving from one to 12 people. Tabulations of cases in California[8] for the 3-year period, 1931-1933, totaled 186 in 13 episodes. In a 6-month period (September, 1934, through February, 1935), Los Angeles County experienced 4 heavy outbreaks involving at least 600 people. We resorted to the feeding of milk cultures of staphylococci to kittens with fairly consistent results. Such work only identified the responsible organism after the outbreaks had occurred. Until a practical and fairly specific test was devised, control measures that might detect possible sources and thus prevent future outbreaks were impossible.

Attempts to develop a satisfactory test have been made by various workers and the results are summarized by Stritar and Jordan[10] who state, "Neither in biochemical, hemolytic nor agglutinative characters is there evidence of homogeneity." This was aggravatingly true in the experience of many laboratory workers. Our first attempts were no exception.

Results obtained in repeated outbreaks gradually provided material for statistical analysis. All foods involved were listed in relation to the predominance in them of carbohydrate, hydrocarbon or protein components with the striking fact that *protein was the only consistent major ingredient of the food involved in every outbreak.* Accordingly we concentrated upon experimental work involving protein. Among tests made were the usual observations for liquefaction of gelatin. However, we soon abandoned the 20°C. incubation and resorted to incubation at body temperature (or over) for the series of tests that followed. Here, again, we met with the experience of others,[10] *viz.*, that liquefaction caused by the same strain varied in degree, incidence and rapidity. Attempts were made to speed up liquefaction.

The basic ingredients of nutrient gelatin, beef extract, peptone, gelatin, were varied in different batches of the medium. Plantings were made from 70 strains of staphylococci isolated from different sources and include pyogenic, septicemic, saprophytic, dermotoxic, hemolytic, lethal and enterotoxic strains. The medium used at present is so specific that it yields positive results with the enterotoxic strains and negative ones with the non-food poison cultures in our own collection.

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[10] Stritar and Jordan, *J. Inf. Dis.*, 1935, **56**, 1.

The majority of the strains were tested several times by feeding kittens, in order to determine whether they were enterotoxic or non-enterotoxic. The test in kittens together with epidemiological findings established the characteristics of the cultures studied.

Beef Extract Gelatin. Dry Difco granular gelatin in an open, wide evaporating dish in an incubator for at least 5 days. In the actual medium use 15% gelatin and 3.0% Difco beef extract. In a double boiler melt these together with the necessary volume of distilled water. In setting of gelatin, variation is influenced by several factors other than the percentage of gelatin. So far we have used the plan described in Wadsworth's Standard Methods in New York State under "Heat Sterilized Gelatin," (page 97). Heat the gelatin gradually until it dissolves. Do not adjust pH. Let it stand without stirring until cooled to 45°C. Raise to 95° to 100°C. for 20 minutes and stir occasionally. Make up the lost weight. Filter through paper. Place 4 cc. in tubes (4x½ inch). Plug tubes with cotton. Sterilize at 15 pounds for 15 minutes. After removal from the autoclave, place at once in ice box. When the gelatin has set, cork and paraffin the tubes to prevent evaporation. 25°C. is the lower limit for melting of the medium ready for use.

When planting, insert loop through the medium to the bottom of the tube. Incubate at 37.5°C. (at this temperature the gelatin naturally becomes liquid). Read results at the end of exactly 24 hours' incubation by placing all tubes in water at 21°C. until the control tubes set.

Any degree of liquefaction evidences a food poison staphylococcus. No liquefaction evidences a negative strain. Some strains completely liquefy while others only slightly affect the surface of the medium. Peptone, mineral salts and carbohydrates in usual amounts seriously retard liquefaction. We hope to definitely establish the essentials in the beef extract necessary for the test. If this is accomplished, then the variables which can occur in different beef extracts as causes of experimental error may be avoided.

8301 P

Preparative Changes Necessitated by a Quantitative Study of Precipitating Power of Pneumococcus Polysaccharides.*

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In an extension of the quantitative study of the precipitin reaction between the specific polysaccharide of Pneumococcus III (S III) and homologous antibody[1] it was found that several S III preparations which precipitated the same amount of antibody from horse serum threw down widely varying quantities of antibody from a rabbit serum. The present preliminary report deals with experiments on S II and S III undertaken to find the cause of variations in the precipitating power of these substances toward homologous rabbit antisera.

1. *Specific Polysaccharide of Type III pneumococcus (S III).* A Chamberland filtrate of 24-hour cultures in phosphate meat infusion glucose broth was concentrated about 12-fold *in vacuo* and precipitated several times with an equal volume of alcohol. The caseous S III was redissolved and separated from protein over the barium salt with barium chloride. Barium was removed by repeated reprecipitation of the S III with glacial acetic acid after addition of sodium acetate. The properties of several lots obtained in this way are given in the table. Preparations in which heat and strong acid were avoided showed far greater viscosity than did samples prepared by the original method.[2] Comparison of viscosities was made in 0.9% salt solution.[3] As will be seen from the table a new preparation precipitates approximately the same amount of antibody from serum as does the original culture filtrate. The new preparations precipitate more antibody from Type III antipneumococcus rabbit serum than those prepared by the original method, although all precipitate about the same amount of antibody from homologous horse antiserum. The sera used were first absorbed with "C"-poly-

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* The work reported in this communication was carried out under the Harkness Research Fund of the Presbyterian Hospital, New York.

[1] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **61**, 563.

[2] Heidelberger, M., Goebel, W. F., and Avery, O. T., *J. Exp. Med.*, 1925, **42**, 727.

[3] Heidelberger, M., and Kendall, F. E., *J. Biol. Chem.*, 1932, **95**, 127.

TABLE I.

Properties of Specific Polysaccharides of Pneumococcus II and III.

Preparation No.	Ash as Na	Nitrogen*	$[\alpha]_D^*$	Neutral equivalent	η rel. 0.10% soln. in 0.9% NaCl	Antibody N pptd. from 1 ml. serum by slight excess S				
						Horse 607	Rabbit B53	Rabbit 350-1	Rabbit 718	
	%	%	Degrees			mg.	mg.	mg.	mg.	mg.
S III										
A 66[1]	0.2	.06	—37.3	350	1.09	1.34	.62	1.12		
S III Ba[2]	0.7	.10	—38.0	351	1.04	1.34	.33	0.86		
S 102[3]	3.4	.43	—30.7	383†	1.18	1.32	.56	1.10		
S 105 B_1[4]	6.3	.22	—32.5	365†	2.64	1.37	.78	1.25		
					1.34[5]			1.22[6]		
S 107B[4]	6.1	.27	—32.8	379†	2.06	1.37		1.18	.80	
								1.17[7]		
105 cult. filtrate									.73	
								Rabbit Pn II RSI		
						Horse B83A	Rabbit R377		heated *in vacuo*	heated in air
S II										
Cu purified	0.0	.16	+52	1000	1.04		.64			
S 80A3	0.1	.20	+61	960	1.04	1.27	.65	0.63		
S 83E[4]	2.2	.46	+53	956†	1.31			0.94	0.94	0.84
Culture filtrate 1						1.27	.87			
” ” 4							.93	1.07	1.02	0.86

*Calculated to the ash-free basis.

[1] Prepared as in Reference 2.

[2] Prepared as in Reference 2 with additional purification with $Ba(OH)_2$.

[3] Prepared by concentrating broth on steam bath, isolating S without the use of strong acid and alkali. S III obtained as the acid sodium salt by precipitating with acetic acid.

†Calculated from the ash content.

[4] Prepared by concentrating broth *in vacuo* and isolating S as neutral sodium salt without use of strong acid or alkali.

[5] After heating 6 hours in boiling water bath.

[6] S III treated with 0.5 *N* NaOH at room temp. for 24 hours.

[7] Amount precipitated after heating S III solution 6 hours at 100°.

saccharide and pneumococcus protein so that the remaining antibody would be mainly anti-S.†

Treatment of the best preparations with 0.5 *N* sodium hydroxide solution[4] at room temperature for 24 hours failed to influence the amount of antibody precipitated from rabbit antisera, nor did heating aqueous solutions in a sealed tube at 100° for 6 hours, although the heat treatment reduced the viscosity markedly. Otherwise, preparations which showed the lowest viscosity gave the lowest values for antibody precipitated from rabbit antisera, a finding consistent with the observation[5] that partial degradation products of S III showing low viscosities as compared with S III precipitated Type III antipneumococcus horse serum but not homologous rabbit antiserum. It would seem that the new S III preparations of exceedingly high viscosity have the longest chain-length, and that the older preparations were partially degraded by the acid used in the purification process and by the initial concentration of the autolyzed culture at 100°. The newer preparations possibly represent the unstable intermediate postulated by Ward[6] as occurring between the native S III antigen and the polysaccharide as then purified.

2. *Specific polysaccharide of Type II pneumococcus (S II).* Similar results have been obtained with S II, and are also summarized in the table. No significant differences were found between an old preparation ("Cu purified") and one made from heated broth without the use of strong acid or alkali (S 80A). After concentration *in vacuo* the S II was precipitated from the culture filtrate by 1 volume of alcohol and extracted with neutralized 20% sodium acetate solution. After several repetitions of these steps treatment with copper acetate at pH 5 precipitated the last of the protein and little S II. The bulk of the S II was precipitated by addition of 5 volumes of glacial acetic acid. The product, S83E, precipitated 50% more antibody from rabbit serum than did the earlier preparations and its viscosity was also much higher.

In the case of S II oxidation appears to be involved as well as hydrolysis when culture filtrates are heated. When either broth or S83E was heated at pH 5.8 at 100°C. *in vacuo* there was little or no change in reactivity toward rabbit serum, but in the presence of air a marked decrease was noted.

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† A statement to this effect was inadvertently omitted from Reference 1.

[4] Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731; Enders, J. F., and Pappenheimer, A. M., Jr., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 37.

[5] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1933, **57**, 373.

[6] Ward, H. K., *J. Exp Med.*, 1932, **55**, 519.

8302 C

Effectiveness of the Shope Papilloma Virus in Various American Rabbits.

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The virus causing cutaneous horns and papillomas in western cottontail rabbits (*Sylvilagus floridanus,* Allen) gives rise to vigorous growths of the same sort in domestic rabbits[1] (Genus, *Oryctolagus*). In animals of both kinds these growths have the immediate character of neoplasms,[2] and in domestic rabbits they frequently progress to cancer.[3] For this reason, the biological range of effectiveness of the virus has a special interest.

Thus far rabbits only have proved susceptible to the virus. A Kansas dealer supplying cottontails with "natural" papillomas states that he has never seen them in any of thousands of jack-rabbits (*Lepus californicus,* Gray); yet in one of 24 he sent us a small but typical growth existed, hidden by the fur. Three mixtures were made of the host's blood serum with various proportions of glycerinated cottontail papilloma extract, and after incubation all were rubbed into the scarified skin of each of 3 domestic rabbits, which received also similar mixtures made with the sera of 4 other jack rabbits of the same lot. Growths developed at every site except where the mixtures with serum from the papillomatous animal had been inoculated.

The "natural" jack rabbit papilloma enlarged slowly for some weeks, when the animal died and it was removed to 50% glycerine. An extract made 36 days later and inoculated into scarified domestic, cottontail, jack and snowshoe rabbits (*Lepus americanus,* Erxleben), 3 of each, produced growths in all. They were indistinguishable from such as are caused by cottontail virus. In 2 further experiments 2 jack rabbits were inoculated with cottontail virus extracts of differing derivations. Growths appeared in 3 of the 4 animals, the fourth dying too soon to be deemed resistant. An extract of the glycerinated papillomatous tissue from 1 of the others gave rise to growths in 2 of 3 domestic rabbits and in 2 jack rabbits.

The findings justify the conclusion that the "natural" jack rabbit papilloma was due to the virus causing the disease in cottontails.

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[1] Shope, R. E., *J. Exp. Med.,* 1933, **58**, 607.

[2] Rous, Peyton, and Beard, J. W., *J. Exp. Med.,* 1934, **60**, 701, 723, and 741.

[3] Rous, Peyton, and Beard, J. W., *J. Exp. Med.,* 1935, **62**, 523.

Two groups of 3 snowshoe rabbits each, obtained from Maine, were inoculated with cottontail virus. All developed growths. An extract of glycerinated papilloma from one was inoculated into 3 snowshoe and 3 domestic rabbits. Again growths developed in all.

The papillomas in snowshoe rabbits were a dirty yellow-gray during the first weeks, and softer and less elastic than those of domestic and cottontail rabbits, appearing somewhat greasy prior to drying into similar jagged masses. The growths in jack rabbits were rubbery and negro-black, with a living base that was notably shallow because the connective tissue cores supporting the papillae were so narrow that drying took place early, to masses which often became high and resembled lava. In their main histological features the growths of all of the various rabbits were alike, however. Sometimes creamy, basal pearls were present amidst the black of the jack rabbit papillomas; and one such growth, the most rapidly enlarging produced with virus from the "natural" jack rabbit papilloma, changed to a "papilloma of the second order" during the 4 months prior to death of the animal from intercurrent causes. This change in domestic rabbits precedes malignancy.

As a rule the papillomas appeared later and grew more slowly in snowshoe and jack rabbits than in domestic rabbits receiving the same inoculum. They regularly yielded active virus, however.

While the Shope virus is pathogenic for jack rabbits, it is far less effective in producing disease among them under natural conditions than in cottontail rabbits occupying the same territory. Possibly, indeed, it is not effective at all; for the papilloma reaching us on a jack rabbit may have resulted from accidental inoculation during the period between trapping and shipping, when cottontails with the growth were also handled. However this may be, the habits of jack rabbits will suffice to explain their general freedom from infection with a virus to which they are susceptible. They are surface creatures of regions without timber, and are solitary as a rule. Whether habits and environmental conditions are together responsible in the case of snowshoe rabbits and eastern cottontails, or whether the disease has not yet reached them is uncertain. New Jersey cottontails (*Sylvilagus floridanus mallurus,* Thomas) are readily infected experimentally, and the induced papillomas yield the virus again.[4] In western cottontails the papillomatosis has been endemic for more than 50 years, and an equilibrium between parasite and host is in process of development, as so often with parasites long affecting one species.[5] Not a few cottontails prove resistant on inoculation with virus, and the spontaneous and induced growths, though often persisting for months or years, are well tolerated and nearly always retrogress in the end. Domestic rabbits, on the other hand, are so highly susceptible that the induced growths frequently progress and kill. But, as frequently happens in the case of parasites reaching new hosts, the disease produced is severe yet the parasitism itself is aberrant,[5] as shown by the fact that from even the most vigorous papillomas of domestic rabbits only an attenuated virus can be recovered, and this infrequently.[6]

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[4] Shope, R. E., personal communication.

8303 P

Certain Factors Determining the Course of Virus-Induced Tumors.

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When retrogressing or enlarging, the chicken tumors due to filtrable causes and the Shope rabbit papilloma conduct themselves like other neoplasms.[1] Their course is determined by a variety of influences. The papilloma is especially suited to the study of these because of its accessibility and discrete character.

Influence of the Initial Virus State. Shope virus from some sources gives rise to progressively enlarging papillomas, and that from others to growths which tend to disappear. Virus artificially attenuated (by heating) causes infrequent, inactive growths which eventually retrogress.[2] In the literature on chicken tumors similar facts find larger illustration.

Host Conditions Affecting the Virus. The blood serum of rabbits carrying the papilloma neutralizes the Shope virus *in vitro.*[3] To ascertain whether the course of the growth is influenced by the circulating antibodies we have tattooed or intradermally inoculated the virus at many points on the sides of 2 comparable groups of domestic rabbits, in addition rubbing it into broad scarified areas on the abdomen of one of the groups, producing in this way confluent papillomatous masses which appeared sooner and enlarged much faster than did the growths on the sides. The latter were all charted at short intervals, and the virus-neutralizing power of the hosts' sera was determined from time to time. Such power was absent prior to inoculation and its rate of development, though differing somewhat from individual to individual, on the whole varied *pari passu* with the increase in bulk of the papillomatous tissue. The power appeared sooner and became much greater in the animals developing large abdominal masses, yet the growths on their sides differed no whit in magnitude and course from those of the control animals. A negative outcome of reinoculation with the virus, on the other hand, proved directly referable to the possession of neutralizing power by the blood.

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[5] Smith, Theobald, Parasitism and Disease, Princeton, 1934, Princeton University Press.

[6] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607; PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 830.

[1] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701, 723, 741.

[2] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

[3] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607.

With the chicken tumors no such categorical test has been feasible. But resistance directed against the virus and ineffective against the cells has long been recognized, as has also the presence of a virus-neutralizing principle in the blood of fowls with growing sarcomas.[4]

Host Conditions Affecting the Papilloma Cells. Many such conditions, local and general, have been reported.[5] The papilloma cells are easily stimulated or depressed. To local tissue conditions and tissue reactions, to dyes and bacterial infection, they respond as do other neoplasms, including the chicken tumors.

Influence of the Cells on the Virus. The virus affects the host organism only secondarily; its first and enduring relation is with the cells. It is pathogenic for epidermis only, and "epidermicity" is of more consequence to it than the rabbit species.[6] Many facts indicate that cells impelled to proliferation by the virus may yet provide a milieu in which this cannot enduringly thrive, the result being a retrogression of papillomas which were vigorous in the beginning. The skins of some rabbits of susceptible breed have certain gross characters which mark them as relatively unsuited to the virus, which produces in them infrequent growths that eventually retrogress as a rule. The tumors resulting from the broadcast inoculation of several large skin patches of a susceptible animal generally behave in the same way, all developing or disappearing together; but when the virus is brought in contact with relatively few cells at each site, by puncture inoculation, the variation in incidence and course of the papillomas is not inconsiderable. The individual cells would seem to offer differing conditions to the virus. Yet still these conditions do not range so wide that some papillomas go on while others of the same host are retrogressing. To see whether the range could be widened experimentally, the scattered papillomas on one side of a number of rabbits were repeatedly injected with Scharlach R., those of the other side serving as controls. The stimulated growths enlarged with extraordinary rapidity, forming great fungoid masses which continued to enlarge after the injections had been discontinued, and they have proved fatal in one instance. The control papillomas remained small and dry, but they progressed also save in 2 instances in which all dwindled and disappeared while the stimulated papillomas continued to proliferate. Weeks later, however, these retrogressed as well, despite a continuance of the dye injections. Some adverse condition of the host or of its cells had eventually become dominant. Host resistance to proliferating neoplastic cells as such, and a persistingly unfavorable virus-cell relationship are both attested causes for the generalized retrogression of chicken tumors.

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[4] Andrewes, C. H., *Lancet*, 1934, **2**, 63.

[5] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701, 723, 741.

[6] Shope, R. E., *J. Exp. Med.*, 1933, **58**, 607; Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701, 723, 741; Beard, J. W., and Rous, Peyton, PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 191.